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THE BIOLOGICAL REACTIONS OF THE VEGETABLE PROTEINS.*

I. ANAPHYLAXIS.

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1. INTRODUCTION.

The biological reactions of the vegetable proteins, with the exception of the phytotoxins, have received scant attention as contrasted with the great mass of investigations upon the proteins of animal origin. This is due in large part to the fact that nearly

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all the investigators in this field have been pathologists, bacteriologists, and clinicians, who were less familiar with vegetable proteins than with animal proteins. In studying these reactions the vegetable proteins offer distinct advantages over those of animal origin, since many of them can be isolated in a higher state of purity. On the other hand, the fact that but few of the vegetable proteins are soluble in water or physiological salt solution is a decided disadvantage in biochemical investigations, which in some cases is insurmountable.

Few references to vegetable proteins are to be found in the literature of immunology, with the exception of the numerous investigations of the phytotoxins, especially ricin and abrin. While these last investigations furnished the first evidence that the animal body would react to the presence of vegetable poisons by the production of antibodies, yet the reaction against the plant toxins with their typical toxin character is a special case, and need not be considered in connection with the general biological reactions of the ordinary plant proteins which are not toxins in the common use of the term. Against the phytotoxins we have the production of true antitoxins; against non-toxic vegetable proteins we may have the production of precipitins, agglutinins, opsonins, and also, under suitable conditions, the anaphylactic or hypersensitive state. There have been a few investigations of the precipitins produced by immunization with various impure extracts of plant tissues, and an even smaller number of experiments which demonstrate that vegetable proteins resemble animal proteins in being toxic to animals which have previously received a small sensitizing dose of the same protein. Concerning agglutinins and opsonins for vegetable substances we can find almost nothing in the literature.

2. HISTORICAL.

a) THE PRECIPITIN REACTION AND VEGETABLE PROTEINS.

Kowarski[1] seems to have been the first to report (July 4, 1901) the observation of precipitins for vegetable proteins. He immunized rabbits with solutions of non-coagulable proteins from wheat flour, and obtained a precipitin for a saline extract of wheat flour. The immune serum also gave a distinct, but less heavy precipitate with extracts of the seeds of peas, rye, and barley, but not with an extract of the seeds of oats. From his observations he suspected that the specificity of vegetable proteins may not be as marked as with animal proteins, but it is probable that the lack of specificity observed was due to the use of too concentrated solutions of the serum and protein.

At about the same time Jacoby[2] reported his observations on the precipitin reaction which occurs when anti-serum for ricin is added to a solution of ricin, but these experiments were not directed to ascertaining the biological reactions of a vegetable protein, as were the experiments of Kowarski. A similar demonstration of the possibility of producing specific precipitins for ricin was reported in the same year by Loewenstein.[4]

Albert Schütze[3] also described the production of an anti-serum for a soluble commercial preparation of vegetable protein ("Roborat") which reacted with this Roborat, but not with a solution of human muscle protein; further tests seem not to have been made. Later Schütze[5] reported some unsuccessful attempts to distinguish between different yeasts (top yeast, bottom yeast, grain yeast, potato yeast) by means of the precipitin reaction.

Ottolenghi[6] immunized with extracts of ergot of rye in physiological salt solution, and obtained a serum which gave a precipitate with aqueous extracts of ergot of rye, but did not react with watery extracts of rye or wheat flour.

The next investigation in this field that we can find recorded was by Bertarelli[7] who investigated beans, peas, lentils, and vetches. He used the non-coagulable proteins in aqueous solution as employed by Kowarski, and also saline extracts, or even fine suspensions of the pulverized seeds. Intravenous injections were found to be dangerous, especially with the bean extracts, possibly because of the agglutinating action of such seed extracts on the erythrocytes. Normal serum gave a precipitate with strong solutions of the proteins which Bertarelli used for immunizing, but only in concentrations of 1 in 10 to 1 in 50, varying with different animals and different proteins. Cautious subcutaneous immunization produced the most active sera, the maximum activity being reached only after continuing the immunizing treatment for six to seven weeks. Immune serum was obtained reacting against extracts of the seeds of *Phaseolus vulgaris* in dilutions as high as 1 in 300 to 1 in 3,000, and this serum reacted with other extracts of leguminous seeds only in concentrations of 1 in 60 to 1 in 100. The results with extracts of pea flour were quite similar. Immunizing against extracts of lentil flour he obtained anti-sera which reacted with this material in dilutions of 1 in 3,000 to 1 in 5,000, but with extracts of other leguminous seeds only in concentrations of 1 in 100 to 1 in 300. Likewise anti-serum against extracts of one sort of vetch or one variety of bean reacted with strong solutions of the proteins of the same genus. Therefore Bertarelli concludes that the precipitins which can be obtained by immunization with vegetable proteins are qualitatively but not quantitatively specific, and that to distinguish a protein by the precipitin reaction, dilutions of not less than 1 in 500 should be used. Attempts were made to differentiate the mushrooms by the precipitin reaction, but without success, for it was extremely difficult to obtain any precipitins whatever by using mushroom extracts and such active sera as were obtained did not react specifically.

Catastini,[10] on the other hand, claims to have obtained specific precipitins for aqueous extracts of various mushrooms, which exhibited quantitative differences with extracts from other than the homologous species. He states, however, that it is quite difficult to obtain precipitins with this material, which we may ascribe to the low content of protein in extracts of these fungi.

Magnus and Friedenthal[45] state that the press juice of *Agaricus campestris* contains but 0.1 per cent of protein, while truffle (*Tuber brumale*) press juice contains only

0.025 per cent, in contrast with press juice from yeast (*Saccharomyces cerevisiae*) which contains 2 per cent of protein (all estimated by the Esbach method). They immunized rabbits, one with each of these three plant extracts, and found that the anti-mushroom serum reacted only with the mushroom extract, the anti-yeast serum reacted well with the truffle extract but not with the mushroom extract, while the anti-truffle serum reacted slightly with the yeast but not at all with the press juice of *Agaricus campestris*. In subsequent papers they described their investigations of precipitin reactions with vegetable proteins, finding, in contradiction to Kowarski, that anti-sera for pea-meal extracts and wheat-flour extracts are mutually specific.[46] They also produced anti-sera for extracts of the seeds of *Ustilago jensenii*, *Mucor racemosus*, *Cocos nucifera*, *Sauromatum*, *Zea mays*, *Oryza sativa*, *Panicum italiacum*, *Triticum sativum*, *Avena sativa*, and *Pisum sativum*, all of which showed definite but limited specificity. Thus, anti-serum for maize flour did not react with extracts of rice flour but did react with extracts of the seeds of *Euchlaena mexicana;* wheat flour anti-serum reacted with extracts of rye and barley flour but not with extracts of oat flour; pea-meal anti-serum reacted with extracts of the seeds of vetch (*Vicia sativa*) but not with those of the seeds of the yellow lupine (*Lupinus luteus*). They consider that precipitins against extracts of vegetable substances are, if anything, more specific than precipitins against soluble animal substances. They also found[47] that precipitins resulting from immunization with any one part of a plant (rye) would react with extracts from all other parts of the same plant, e.g., seeds, leaves, and roots.

Gasis[8] in 1908 produced precipitins by immunizing with the non-coagulable proteins, obtained by boiling and filtering watery extracts of rye, rice, and various beans. He found these to show a high degree of specificity, contrary to Kowarski and Bertarelli, and states that if quantitative relations were taken into consideration closely related species could be distinguished; e.g., *Phaseolus vulgaris* could be distinguished from *Phaseolus multiflorus*. The proteins of rice seeds, however, seemed to be less specific than the proteins of the seeds of other cereals or of those of the seeds of leguminous plants.

Relander[9] published a preliminary report in the same year stating that he had found it possible to distinguish different varieties of grains and vetches by their precipitin reactions, but gave no details as to the materials or methods used. We have been unable to find any subsequent reports from this observer.

Magnus[48] properly criticizes the observations of Gasis on the ground that they were not sufficiently controlled, for many plant extracts, and especially those of the cereals, give precipitates with normal sera. He finds that the extract of rice flour produces especially heavy precipitates with normal serum, being followed closely by extracts of the flour of maize and oats, while extracts of rye, barley, and wheat flour produce practically no such precipitates. He states that in order to test such plant extracts as give precipitates it is necessary to first remove the precipitating elements by saturation with normal serum and filtration, then using the filtrate against the immune serum. Taking into account these and other sources of error, Magnus finds that the degree of immunization determines the range of reaction. If, for example, an animal is immunized but a short time with extracts of the seeds of one of the cereals, its serum may precipitate only the extract of the same species; a little later in the course of immunization, precipitates will appear for extracts of closely related species, and progressively a wider and wider list of cereals will react, until finally precipitates may be obtained with any and all *Gramineae*. Nevertheless, even with this extreme degree of immunity no reaction will be given by the serum with extracts derived from plants not belonging to the *Gramineae*. As the order of appearance of reactions with heterologous extracts is always the same, it is possible in this way to secure a standard for estimating the relationship of plants by biochemical means.

Magnus[49] finds that the specificity of the precipitins resulting from immunization with physiological saline solutions of grain products is sufficiently marked to permit of detection of adulteration and mixing of such materials. In order to do this it is necessary to take into account quantitative as well as qualitative differences and in the case of closely related grains, differentiation can be secured only through the use of the saturation process. By this means Magnus was able to detect the presence of 3 per cent of wheat flour in rye flour, and $\frac{1}{2}$ of 1 per cent of "castor meal" (*Vicia faba*) in wheat flour.

Dunbar[50] was unable to obtain precipitins by immunizing with the pollen of rye, maize, solidago, and ambrosia, in which respect pollen differs from other plant structures. A complement-binding serum was obtained, however, which was peculiar in not reacting with other parts of the plant; as similar results were obtained with spermatozoa of fish and animals when contrasted with the flesh and serum of these animals, he considers that the sex cells are specifically distinct from all other elements of the organism, a view which is opposed by Magnus and Friedenthal.[51] Precipitins for extracts of rye leaves and grains were found by Dunbar to react more strongly with the homologous than with the heterologous extract.

Recently Wilenko[15] has studied the precipitation which occurs when extracts of various vegetable materials are added to sera or other protein solutions. This phenomenon was first observed by Kraus[16] who obtained a precipitate when ricin was added to sera of various origins, but only when the sera were from animals whose red corpuscles are agglutinated by ricin. Similar results have been obtained with abrin, and with physiological saline extracts of beans (*Phaseolus*), lentils (*Ervum*), peas (*Pisum*), and vetches (*Vicia*), by Landsteiner and Raubitschek,[17] and with *Datura stramonium* by v. Eisler and v. Portheim.[18] Wilenko[15] found that all normal sera gave precipitates with ricin,* abrin, and crotin, and with extracts of the seeds of oats and maize, bird sera reacting most strongly and carp serum the least. Solutions of hemoglobin, serum albumin, and egg albumin all gave precipitates with these same plant extracts, and even one plant extract may cause precipitation in another.† Excess of serum prevents the formation of a precipitate, while heating the plant extracts to 80° C. destroys their precipitating action. Solutions of ricin and extracts of maize absorb complement, when added to guinea-pig serum. These phenomena Wilenko ascribes to "normal precipitins" present in the plant extract, which he assumes, without advancing any proof of his contention, to be entirely analogous to the immune precipitins; neither does he prove that the reactions observed depend upon the proteins contained in his saline extracts of the seeds, rather than upon other extractive substances also present.

Raubitschek[21] immunized rabbits against saline extracts of lentils and beans, and found that the serum of these rabbits would inhibit the agglutination of red corpuscles by such extracts, but would not inhibit agglutination by ricin; therefore it would seem probable that the natural phytagglutinins are each characterized by a specific antigen. He mentions, as a preliminary report, that these anti-sera have a specific inhibiting effect upon the development and growth of the seeds of the species furnishing the extracts used for immunizing. De Angelis[19] claims that specific precipitins can be obtained by immunizing with vegetable dyes.

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* Osborne, Mendel, and Harris, in their experiments with a highly purified ricin (*Am. Jour. of Physiol.*, 1905, 14, p. 259), found that the serum of a rabbit immunized with this ricin yielded a precipitate with the ricin, but the serum of a normal rabbit yielded *no* precipitate under the conditions of the experiment unpublished observation).

† In this connection we may point out that Hardy's experiments with serum globulin indicate that this protein has more pronounced acid than basic properties, whereas Osborne[54] found that edestin is more basic than acid, and it is known that when proteins of opposite reaction meet they commonly form precipitates.

b) ANAPHYLAXIS WITH VEGETABLE SUBSTANCES.

Little as is known concerning the behavior of the vegetable proteins as antigens in the precipitin reaction, their behavior in the anaphylaxis reaction has been even less considered. Rosenau and Anderson[11] observed in a brief series of experiments that guinea-pigs which had been sensitized with a watery extract of peas reacted violently (3 in 5 died) to a subsequent injection of the same material, establishing that vegetable extracts resemble animal and bacterial proteins in this respect. They performed no experiments to test the specificity of the reaction with pea extracts, nor with the extracts from yeasts and various bacilli which they found to sensitize guinea-pigs to the homologous extract. In a later paper they reported unsuccessful attempts to secure satisfactory reactions with edestin and excelsin dissolved in 10 per cent NaCl solution, using for the second or intoxicating dose 3 c.c. of a 1 per cent solution of edestin subcutaneously. Their failure in these last experiments we may ascribe to several different factors: first, the small quantity of relatively insoluble proteins used in the second dose; second, the subcutaneous injection, which is the least favorable route for producing the anaphylactic intoxication; third, precipitation of the protein because of diffusion of the salt which served as solvent for the protein, for Mendel and Rockwood[55] found that solutions of edestin in 0.5 per cent sodium carbonate, or of excelsin in 0.9 per cent sodium chloride, yield precipitates of unchanged protein when injected intraperitoneally; and fourth, the simultaneous injection of so much NaCl, for it has been found by Friedmann and Hartoch[13] that by injection of NaCl solution into either actively or passively immunized guinea-pigs the anaphylaxis reaction can be prevented; this inhibition of the reaction they ascribe to the fact that NaCl in sufficient quantities prevents the binding of complement.

Having observed that gelatin will not cause the anaphylactic reaction, Wells[14] suggested as an explanation for this failure the deficiency of aromatic radicals which is characteristic of gelatin, and for comparison investigated the behavior of zein and gliadin, zein being rich in tyrosine and phenylalanine, although devoid of tryptophane, while gliadin is poor in tyrosine and phenylalanine, although it contains tryptophane. Zein was found to actively intoxicate guinea-pigs sensitized with small quantities of zein, fatal reactions being frequently obtained. With gliadin, reactions were sometimes but not always obtained, and in only one of twenty experiments was a fatal intoxication observed.* Animals sensitized with zein did not react to gliadin, or conversely. These were the first, and until now the only recorded successful anaphylaxis experiments with pure proteins isolated from vegetable materials.

Raubitschek[21] sensitized rabbits with saline extracts of lentils and beans, and obtained severe, often fatal reactions, upon subsequent intravenous injections of the homologous extract; with the heterologous extract, reactions were also obtained, but usually less severe. The results secured by such intravenous injections are complicated by the strong hemagglutinating property of such extracts and hence the above experiments have relatively little significance.

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* Later experiments with a more soluble preparation of gliadin have given more severe reactions.

Pozerski[41] obtained a form of anaphylaxis reaction by injecting guinea-pigs subcutaneously with papain in repeated small doses.* Uhlenhuth and Weidanz[52] injected into guinea-pigs raw linseed oil, commercial ricin, and cocoa butter, and later found them sensitive respectively to extracts of flax seeds, castor beans, and cocoa beans.

Karasawa[56] found that typical anaphylactic reactions are produced in guinea-pigs with saline extracts of ground rice, beans, and wheat; sensitization was by subcutaneous injection and the intoxicating dose was given intravenously. Passive sensitization was produced by injecting the serum of sensitized guinea-pigs or rabbits into normal guinea-pigs. One experiment showed that a dog reacted to rice extract with a sharp fall in blood pressure and decreased coagulability of the blood, these phenomena being characteristic of anaphylaxis in the dog. With these extracts a well-marked specificity was shown, guinea-pigs sensitized with rice extract not reacting to saline extracts of sago, rolled barley, wheat flour, beans, or maize; guinea-pigs sensitized with bean extracts not reacting to extracts of lentils, green peas, nut extracts, yellow peas, or "large beans." Only one experiment was performed with each of these substances, and the author failed to determine how much protein was present in the solutions used.

Schern[59] also records the use of the anaphylaxis reaction for the detection of the presence of small amounts of poisonous seeds mixed with food stuffs. He studied extracts of rye bran, peanut flour, and sesame-cake meal, which were contaminated or adulterated with castor-bean meal, cockle, charlock, and ergot.

c) AGGLUTININS AND OPSONINS.

The possibility of stimulating the formation of specific agglutinins for vegetable matter has been demonstrated by Cao[20], who found that by immunizing dogs with starch grains from different sources, it was possible to obtain sera agglutinating starch granules, the reaction showing definite specificity. On the other hand, Porges[43] was unable to increase the opsonic power of rabbit's serum for starch granules by repeated injections of starch.

d) CONCLUSIONS.

It is evident, from the above review of the literature, that the biological reactions of the vegetable proteins have been very inadequately considered, especially in regard to the behavior of isolated preparations freed as far as possible from other substances present in the crude extracts used in most recorded investigations. It is our purpose, in this and subsequent papers, to report investigations performed with vegetable proteins which have been purified as far as the means at present available will permit, and especially with proteins which have been studied as to their content of amino-acids. By using such material it is hoped to put the immunological reactions upon something more nearly approaching a chemical basis than is possible by experiments with sera, egg white, and extracts of tissues and plants, all of which are of complex composition. It is probable that many of the vegetable proteins can be isolated in a purer condition than most of the animal proteins because they form a relatively large part of the reserve food material of seeds, in which they are associated chiefly with non-protein substances from which they can frequently be easily separated. These proteins consequently offer a better opportunity for a study of the biological relations of the proteins themselves than do the animal proteins, about the preparations of which usually much uncertainty exists as to their complete separation from other substances which may contribute, in part or in whole, to the results obtained by their use.

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* Wechselmann[32] considers as a form of anaphylaxis the hypersensitive condition which workers in satin wood (*Fagara flava*) acquire, resulting in erysipelatoid inflammation whenever the skin comes in contact with even a particle of the wood. The active agent seems, however, to be an alkaloid, and therefore this particular form of hypersensitivity is presumably not the same as hypersusceptibility to proteins.

As a matter of convenience we have begun our investigations with a consideration of the anaphylaxis reaction as induced by vegetable proteins. Before describing our experiments it is necessary to discuss the degree of specificity exhibited in the anaphylaxis reaction as shown by previous investigations with proteins of animal origin.

3. SPECIFICITY OF THE ANAPHYLAXIS REACTION.

As yet the limits of specificity of the anaphylaxis reaction are not so well defined as with the precipitin and the complement fixation reactions. It is known that the precipitin reaction becomes less and less differential the more closely related are the sources of the proteins which are compared, and with those from very closely related species precipitins may show only quantitative differences. According to Bauer[23] the complement fixation reaction is much more specific than the precipitin reaction, and Brück[44] has claimed that he can distinguish different species of monkeys from one another by this means, and even that different secretions of body fluids of the same species can be thus distinguished. While this last statement failed of complete confirmation by Bauer, he found that the milk and blood of the same species can be differentiated by the complement fixation reaction.

In their first communication upon the subject of anaphylaxis, Rosenau and Anderson[24] reported a number of experiments made with the object of learning the degree of specificity of the reaction. They found in general that guinea-pigs sensitized with 1/250 c.c. of the serum of one species would in some instances show slight symptoms when 4 to 6 c.c. of the serum of some other species were given intraperitoneally, but as a rule these symptoms were not of any significance as compared with the violent reactions which occurred in the same animals when they were subsequently injected with the serum of the same species as had been used for the first injection. They therefore concluded that the reaction is specific if the degree of reaction is taken into account, that is, it is quantitatively specific. Recent work by these investigators and many others has shown that the degree of specificity is perhaps even more marked than they had at first indicated, although the reports have not been entirely unanimous. One source of error has been pointed out by Uhlenhuth,[25] Pfeiffer,[26] and Thomsen;[27] namely, that some foreign sera are more or less toxic to normal guinea-pigs, and that unless carefully controlled such toxic effects may be interpreted as anaphylactic intoxication. So far as the specificity of the anaphylaxis reaction for foreign blood and serum is concerned, the results obtained by Thomsen[27] are most conclusive. Wishing to decide the possibility of medico-legal application of the anaphylaxis reaction for the determination of the nature of blood stains, he performed careful experiments in which the guinea-pigs were sensitized with small quantities of material obtained from dried blood drops, and found it possible to determine accurately the species of animal from which the drop of blood had been derived, by ascertaining with what sort of serum the sensitized animal would react. He found also, in agreement with the results of Rosenau and Anderson, that injection of a heterologous serum into a sensitized guinea-pig does not render it refractory to a subsequent dose of the homologous foreign serum unless the animals supplying the foreign sera are very closely related, e.g., sheep and goat. Neufeld[28] claims that human and monkey (*Affe*) blood cannot be distinguished by the anaphylaxis reaction, and it is well known that they react with the same precipitins. Trommsdorff[29] states that rat blood and mouse blood cannot be distinguished by this means, although by both the precipitin test and the complement fixation reaction they can be sharply differentiated, and more recently Pfeiffer and Mita[58] have claimed by using the fall of temperature as the index of reaction to distinguish rat blood from mouse blood.

On the other hand, a very high degree of specificity is claimed for the anaphylaxis reaction, at least in certain cases, by Thomsen,[30] who states that it is possible to differentiate the hemoglobin and the serum of the same blood by the anaphylaxis reaction, as guinea-pigs sensitized against foreign red corpuscles will not react with the serum of the same blood, or conversely.

With milk, interesting relations have been observed. Rosenau and Anderson[12] found that guinea-pigs sensitized with human milk or dog's milk did not react with cow's milk, but that when sheep's milk was used for sensitizing, a severe reaction might be obtained with cow's milk; this indicates that, as with serum, heterologous reactions are more likely to occur when the milk is from closely related species. Wells[14] found slight but uncertain reactions when serum and milk of the same species of animal were used against each other for the sensitizing and the intoxicating doses, but the number of experiments performed was too small to give significant results. The same criticism may also be applied to the observations of Uhlenhuth and Haendel[31] who claim that guinea-pigs sensitized with bovine serum will react with cow's milk, and conversely, which they contrast with the finding that precipitins for cow's milk react with bovine serum whereas precipitins for bovine serum react but feebly with cow's milk. Besredka[32] states that animals sensitized with cow's milk react also with goat's milk. Similarly Pfeiffer and Mita[42] obtained some slight reaction when goat's milk was injected into guinea-pigs that had been sensitized with cow's milk. Of particular significance is the observation of Rosenau and Anderson[33] that a guinea-pig sensitized with milk, egg white, and horse serum, either together or separately, will react with each of the three fluids given in series. The blood of such a triply sensitized animal when injected into other animals will likewise render them passively sensitive to all three.[12] The results of Gay and Southard,[34] indicating that any one of these fluids may sensitize to the other two, are difficult of explanation in the light of many other observations to the contrary.

Uhlenhuth and Haendel[31] found that animals sensitized to chicken's serum will react to chicken's egg white, or yolk, or hemoglobin; if sensitized to chicken's hemoglobin they react to chicken's serum, and if sensitized to stroma of chicken's corpuscles they react to egg white or yolk. In all these heterologous reactions, however, there is not developed a refractory condition to the homologous protein, which indicates that there are probably separate as well as common sensitizing and intoxicating substances in even these closely related materials.

Rosenau and Anderson summarize their conclusion concerning the specificity of the anaphylaxis reaction as follows: "The anaphylactic reaction in the guinea-pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species or between proteins of widely different origin." Subsequent developments have not materially modified the validity of this estimate.

Anaphylaxis with bacterial substance, which is perhaps more closely related to anaphylaxis with vegetable proteins, has also been found to exhibit a considerable degree of specificity. The pioneer demonstration by Rosenau and Anderson[11] that the anaphylactic reaction could be produced with extracts of bacterial cells has been repeatedly confirmed, and efforts made to determine the degree of specificity. While Delanoe[35] has claimed that there is no specificity whatever in bacterial anaphylaxis, the positive results of Holobut and Kraus seem to establish the existence of a marked degree of specificity both with active and passive sensitization. Holobut[36] found that specific reactions could be obtained with *B. typhosus*, *B. coli*, and cholera vibrios, and although interaction between colon and typhoid bacilli can be obtained this is to be looked upon as a group reaction, such as is obtained with agglutinins and precipitins. Kraus and Amiradzibi[37] also found that passive anaphylaxis was specific, and that the active poison of anaphylaxis could be produced only when the serum of sensitized animals was mixed with the homologous bacterial substance. Unfortunately, in all these experiments with bacteria the small number of experiments and the insufficiency of controls leaves the results in far from a satisfactory state.

As special cases of specific anaphylaxis should be mentioned the observations of Rosenau and Anderson upon anaphylaxis with placental substance, and the experiments of Kraus and others upon the specificity of anaphylaxis with crystalline lens extracts. The former authors[12,38] found that guinea-pigs could not be sensitized with blood from fetal guinea-pigs, whereas a certain degree of reaction could be obtained by sensitizing and reinjecting with extracts of guinea-pig placenta. With the protein of the crystalline lens peculiar relations as to specificity have been shown by Uhlenhuth with the precipitin reaction, which indicate that the precipitinogen of this protein is devoid of species specificity but that it has a specific relation to the crystalline lens. These ideas are based upon the well-corroborated observation that the precipitins produced by immunizing with lens substance will react with extract of crystalline lens from animals of most diverse species, but will not react with the serum or other proteins of even the homologous animal. Kraus[39] found a similar specificity when anaphylaxis experiments were performed with crystalline lens extract. Thus, animals sensitized with horse lens would react with extracts from the lens of the ox but would not react with horse serum; likewise, sensitization with ox serum did not make animals reactive to ox lens. Andrejew[40] has obtained similar results, but a critical consideration of his protocols does not indicate that the reaction is as strikingly free from species specificity as his conclusions indicate; a very considerable percentage of the heterologous reactions were negative, while the homologous reactions were constant and violent. Pfeiffer and Mita[42] also found some evidence of the lack of species specificity of lens proteins, but they obtained much stronger reactions with the homologous than with the heterologous protein. They corroborate the lack of reaction to serum of animals sensitized with the homologous lens extract. These authors used as their measure of reaction the severe fall in temperature which takes place during the anaphylactic shock, and with this as an index rather than the symptoms of intoxication, they claim that the anaphylactic reaction is highly specific, distinguishing even between hemoglobin and serum of the same species. Uhlenhuth and Haendel[31] have made the remarkable observation that so highly specific is the lens protein that guinea-pigs can be sensitized to guinea-pig lens substance, and they even could sensitize a guinea-pig with the lens of one of its eyes and obtain a reaction by injecting later an extract of the lens of the other eye.

Taking the above-mentioned observations together it would seem that the anaphylaxis reaction, as it is usually observed, has much the same limits of specificity as the precipitin reaction and other immunity phenomena. That is, with some substances marked group reactions are shown, and with material from closely related species quantitative differences are not always constant; on the other hand, with some substances differences within a single species can be detected by the anaphylaxis reaction, e.g., serum and hemoglobin from the same blood, lens protein and serum protein, and also milk and serum from the same species. Taking the published results as they stand it would seem that the specificity of the anaphylaxis reaction is somewhat less sharply defined than the specificity shown by the precipitin reaction and the complement fixation reaction; however, it is possible that this is largely ascribable to the relatively coarse index of reaction furnished by the symptomatology of an intoxicated guinea-pig, as contrasted with the delicacy of observation possible with the precipitin and the complement fixation reactions. There are sources of confusion and error in the observation of symptoms, because of the possible intoxication by the foreign protein independent of anaphylaxis, differences in the rate of absorption, and sundry accidental injuries and intoxications that may complicate the results. Another ever-dangerous source of error lies in the infinitesimal dose that may cause sensitization, which, in the case of egg albumin, has been estimated as one-twenty-millionth of a gram.[14]

Such quantities of foreign proteins may be readily introduced unintentionally, and unless special mention is made of precautions taken to avoid such contamination one is justified in being skeptical of heterodox positive results. The hereditary transmission of sensitization adds a still further complication. It is probable that when all investigators appreciate fully these sources of difficulty, and when more accurate and more objective measures of anaphylactic shock are introduced, such as the fall of temperature, and perhaps other yet unknown objective standards, the anaphylaxis reaction will be found to be fully as specific as any other immunity reaction.

4. INFLUENCE OF DIET UPON ANAPHYLAXIS REACTIONS.

One factor which must come into consideration in studies of the biological reactions of the vegetable proteins is the influence of diet—a factor of less concern when animal proteins are injected into guinea-pigs or other graminivorous animals. It has been shown by several investigators that proteins fed to experimental animals are absorbed in greater or less amounts so little changed that they can be detected in the blood by the precipitin test, and are capable of acting as antigens, since animals so fed with unusual proteins develop precipitins for these proteins. Rosenau and Anderson also found that guinea-pigs fed upon horse serum and horse meat became sensitized to these proteins.

We have found that feeding of cow's milk, dried ox blood, and dried egg white daily for 3 to 8 weeks to guinea-pigs renders them sensitive to the corresponding proteins. This sensitization is specific, since animals fed upon ox blood and reacting to bovine serum did not react to dog serum, horse serum, human serum, or egg white, showing that during or before absorption the food protein that acted as anaphylactogen did not lose its specificity. We found that after two month's feeding the animals reacted less strongly than after one months' feeding, but we have not yet been able to carry on the feeding long enough to learn if complete immunity to an animal protein can be obtained in this way.

One set of experiments with zein, a protein obtained from Indian corn (*Zea mays*), gave results which indicate that there may exist such an immune or refractory condition to a vegetable protein which is taken daily in the ordinary food of the guinea-pigs. It was noticed in a series of experiments performed one autumn with zein upon a certain lot of guinea-pigs that only slight or negative reactions could be obtained. During the previous winter and spring strong reactions had been obtained with the same preparation of zein with the guinea-pigs from the same breeder, and investigation showed that during the summer and autumn the breeder had been feeding his stock largely upon green corn, ears and all. This suggested that the daily ingestion of corn proteins had made the animals immune to zein, and experiments were performed to determine this. Young guinea-pigs, of approximately equal ages, just weaned, were obtained from another breeder who had never fed any form of corn product to his stock. To part of them moistened corn meal was given daily for six weeks as the chief food, while the other lot was fed upon oats, carrots, and hay. The animals then received each a sensitizing dose of zein, and three weeks later a second dose of the same protein; the animals which had been fed on corn meal showed no reaction whatever, while those that had not been thus fed all reacted to the zein. (These experiments are given in detail in Table 14, experiments 1–19.)

Experiments with oat proteins were also undertaken, because all our animals had been fed on oats since birth. Ground oats were extracted with 1 per cent KOH, and the filtrate neutralized with acetic acid, which threw down a considerable precipitate of proteins. These proteins, after being washed and dried, were dissolved in 0.1 per cent NaOH when used for the experiments. When injected into normal oat-fed guinea-pigs in 0.1 gm. doses this protein seemed to be somewhat toxic, for the animals after one hour or longer seemed slightly ill, inclined to lie down, and the coat became rough. The degree of intoxication varied much in different animals, some showing practically no symptoms. The effects were not very similar to the typical anaphylactic intoxication, in respect to the long latent period after injection, and the absence of cutaneous irritation and respiratory difficulty. Several guinea-pigs were then given doses of this oat protein varying from 0.001 to 0.1 gm. and after 15 days a second dose of 0.1

gm. was given. None showed a typical anaphylaxis reaction, although all became more or less ill after one hour or more as did the normal animals on receiving the first injection.

From these experiments it would seem that guinea-pigs may become sensitized to foreign animal proteins upon which they are fed, so that they react to the same protein. When raised upon corn and oats, however, they do not seem to become sensitized to the proteins derived from these grains, but rather to be immune, so that no satisfactory anaphylactic reaction can be obtained by two properly spaced injections. Whether this difference between the vegetable and the animal proteins depends upon the character of the proteins themselves, or upon the fact that the vegetable proteins have been taken in the food more abundantly and for a longer time than the animal proteins, thus favoring the establishment of a condition of immunity, is a matter that requires more investigation. From the results so far obtained, however, it seems probable that animals which have been rendered sensitive to a certain animal protein by feeding with this protein for a few days or weeks may become refractory to it when fed for a longer period. This suggests that it is the protracted and abundant feeding of vegetable proteins which accounts for the refractory condition observed in normal guinea-pigs when the anaphylaxis reaction is tried with vegetable proteins which are constituents of their customary food.

5. DESCRIPTION OF PREPARATIONS USED IN PRESENT WORK.

In the following pages we give some results of our investigations of the anaphylactic reaction obtained with preparations of several of the more extensively studied vegetable proteins. Since there is strong evidence that the anaphylactic reaction is in fact caused by proteins and not by some associated substance it is of fundamental importance to begin its study with the most carefully purified and chemically best defined preparations of the proteins that can be obtained.

Nearly all the published data respecting the anaphylaxis reaction relate to experiments with naturally occurring fluids which contain several protein substances, as well as many other substances of known and unknown character. The only experiments as yet described with so-called pure preparations of proteins have been made with crystallized ovalbumin, ovomucoid,[60] crystallized edestin from the hemp seed, zein from maize, gliadin from wheat, and serum proteins separated from one another by fractional salting-out processes. Since these experiments plainly indicated the importance of greatly extending their range we have undertaken the work described in the following pages.

In regard to the chemical individuality of the preparations used in our experiments we have no conclusive evidence, for with the means at present available no preparations of proteins can be obtained which in this respect satisfy the requirements of the organic chemist. The preparations, however, represent those products which for the present we have to treat as chemical individuals.

That each of these preparations was completely separated from *all* the other proteins of these seeds is not certain. It is probable that those from the leguminous seeds (*Pisum*, *Vicia*, *Soja*) contained small quantities of some of the other proteins of the seed from which they originated, for the separation of these proteins presents peculiar difficulties which makes the production of absolutely pure preparations practically impossible. That these separations in the case of the preparations from the leguminous seeds were in fact imperfect is indicated by the results of our present experiments, as is later set forth in our discussion of the specificity of the reactions.

Most of the preparations of the proteins from the oil-seeds (*Ricinus*, *Cannabis*, *Cucurbita*, *Linum*, *Cocos*, *Bertholletia*) probably represent the best defined and, from a chemical standpoint, the purest protein preparations used in this work. Most of these were crystalline, and were obtained under conditions which our experience has shown readily yield products of a relatively high degree of purity.

The proteins from the cereals (wheat, rye, barley, maize) which were used for our anaphylaxis experiments were all obtained from solutions containing 75 per cent or more of ethyl alcohol. The method employed was such as to make it improbable that these preparations contained more than minute traces, if any, of the other known protein constituents of the seed, and we have every reason to believe that these are among the best characterized of any proteins at present available for such studies.

The preparations from these three groups of seeds used in our present work represent not only biologically but also chemically different types of proteins. Those from the oil-seeds are globulins which dissolve readily in solutions of neutral salts and are in most cases precipitated by dialysis from such solutions in well-formed microscopic crystals. They are known to be characterized by a high content of basic nitrogen. With the exception of the globulins from the flax seed and cocoanut, which have not yet been examined in this respect, this basic nitrogen has been found to belong mostly to arginine. The proportions of the other amino-acids yielded by hydrolysis present no striking features. Differences have been recognized which leave no doubt that each of these proteins is chemically distinct, a conclusion now supported by the anaphylaxis reaction.

The proteins from the leguminous seeds which we used are likewise globulins but have never been obtained in crystals. They resemble one another in their solubility relations which are distinctly different from those of the proteins from the oil-seeds. These differences are difficult to describe but are readily appreciated by those who work with them. While the oil-seeds appear to contain but a single globulin, most of the leguminous seeds contain two or more. It is therefore difficult to make preparations from these seeds which offer the same assurance of purity as those from the oil-seeds. The proportion of the several amino-acids obtained by hydrolyzing these leguminous proteins is much the same for each, and very similar to that yielded by most animal proteins. Nevertheless, sufficient differences have been found between them to justify the assumption that each is a chemically distinct protein.

The cereal proteins used have been limited to those soluble in alcohol of 75 per cent or stronger. These are thus not only sharply differentiated from the other proteins used, but from all other known proteins of either animal or vegetable origin. They are also characterized by yielding no lysine or glycocoll, very little arginine and histidine, and from 26 per cent (zein) to 42–43 per cent (hordein and gliadin) of glutaminic acid.

It is thus evident that the different proteins used in our experiments cover a wide range in properties, constitution, origin, and method of preparation and the results obtained with them should afford a sufficiently extensive review of the field under examination to serve as a substantial basis for further work.

In making most of these preparations clean seeds, apparently free from those of other species, were used, but we are not certain that a very small number of foreign seeds were not, in fact, present in the samples ground. We are also not certain that very slight contaminations with proteins from other seeds may not have occurred through imperfect washing of some of the bulky apparatus used in their preparation or from the dust of other preparations being blown about the laboratory in which large quantities of proteins of all sorts had been made and kept for several years.

Only the preparation of edestin, used in the experiments here described, came fully up to the requirements demanded by these considerations.

As will be seen from the following detailed description of the several preparations used, sufficient care was taken in making most of them to insure products of relatively high purity. We considered them to be suitable for a preliminary study of the anaphylaxis reaction, and this belief is supported by the results obtained with them, although in obtaining final data, especially in regard to the specificity of the reaction of the individual proteins and the relative anaphylactic power of each, preparations made with special precautions must be used. Those unfamiliar with the properties and character of these different vegetable proteins will find an extensive review of what is at present known of them in a paper recently published by one of us (O.) in *Ergebnisse der Physiol.*, 1910, 10, pp. 47–215. A detailed account of the methods used in preparing the different vegetable proteins has been given by the same author in Abderhalden's *Handbuch der biochemischen Arbeitsmethoden*, Berlin, 1909.

Some of the results which we have thus far obtained indicate points to which special attention must be directed. The data here recorded must, therefore, be regarded as of a preliminary character from which only a general survey of the field has been obtained. It is our present intention to greatly extend the scope of this work and to confirm our results by many properly controlled experiments so that definite conclusions finally may be obtained.

Legumin, pea.—A large quantity of a preparation of legumin, which had already been freed from nearly all of the vicilin with which it was originally associated, was dissolved in 1 liter of water containing 76 gms. of $(NH_4)_2SO_4$. An equal volume of a saturated solution of this salt was then added gradually and then enough more crystals of $(NH_4)_2SO_4$ to make the solution $\frac{6}{10}$ saturated. The large precipitate which separated was filtered out and dissolved in 2 liters of water, the adherent sulphate being sufficient to bring nearly all of the protein into solution. An equal volume of a saturated solution of $(NH_4)_2SO_4$ was next added and then 250 gms. of crystals of this salt, thereby making the solution approximately $\frac{6}{10}$ saturated. The precipitate thus obtained was filtered out, freed from most of the adhering solution by pressing between filter papers, and redissolved by suspending in 4 liters of water and adding crystals of $(NH_4)_2SO_4$. Enough of this salt was then added to make the solution $\frac{7}{10}$ saturated. The legumin which was so precipitated was collected on a filter and when freed as far as possible from the solution it was dissolved in 10 per cent NaCl solution and filtered through a pulp filter. The solution was then dialyzed in running water for 4 days. The dialysis precipitate was washed first with water, then with dilute alcohol, digested with absolute alcohol, and dried over sulphuric acid. When this preparation was dissolved in NaCl solution it remained perfectly clear on boiling, thus indicating absence of vicilin. It also gave no Molisch reaction, from which we conclude that it contained no nucleic acid compounds or carbohydrates. This preparation can be considered to be as free from vicilin or other proteins of the pea as it is possible to make one with any reasonable amount of care.

Legumin, vetch.—The finely ground seeds were treated with three times their weight of 5 per cent NaCl solution, to which was previously added a quantity of cold saturated baryta solution just sufficient to neutralize to litmus the acid reaction of the meal. The extract was filtered perfectly clear and then dialyzed for 4 days until free from chlorides. The legumin which had separated on dialysis was then filtered out, redissolved in 5 per cent NaCl solution, and the solution which resulted filtered through paper pulp and again dialyzed for 4 days. The precipitate produced by dialysis was washed carefully by suspending in distilled water, all lumps being broken up by passing the suspension through fine bolting cloth. After sucking the legumin as dry as possible it was dehydrated by treating with absolute alcohol, digested with dry ether, and dried over sulphuric acid. Since legumin is the only protein that is precipitated by dialysis from extracts of the vetch the above process is sufficient to separate it from all but minute traces of legumelin and proteose which are also present in extracts of this seed.

Vicilin.—Finely ground pea meal was extracted with 10 per cent NaCl solution and the extract was filtered perfectly clear and dialyzed in running water until free from NaCl. The precipitate produced by dialysis was dissolved in $\frac{1}{10}$ saturated $(NH_4)_2SO_4$ and enough solid $(NH_4)_2SO_4$ added to the solution to make it $\frac{65}{100}$ saturated.

The precipitate was filtered out and the filtrate was saturated with $(NH_4)_2SO_4$. The precipitate which resulted was freed, as far as possible, from the solution, dissolved in 10 per cent NaCl solution, and, after filtering clear, the solution was dialyzed till nearly free from NaCl. The precipitate, which should contain any legumin still present, was filtered out and the solution treated with a current of CO_2. The precipitate thereby produced was dissolved in dilute NaCl solution and dialyzed until the globulin was precipitated. This was then washed with water and with alcohol and dried over H_2SO_4. The preparation thus obtained had the ultimate composition of vicilin but whether or not it was entirely free from any legumin is not certain. It was considered to be pure enough for preliminary experiments.

Vignin.—This preparation was made by extracting ground cow-peas with 5 per cent NaCl solution, filtering the extract perfectly clear and dialyzing for three days. The precipitate produced by dialysis was filtered out, suspended in a measured quantity of water, and dissolved by adding a weighed amount of NaCl. The solution was then filtered perfectly clear and the vignin precipitated by diluting with water until the solution contained 1 per cent of sodium chloride. The precipitate which resulted was allowed to settle, the supernatant solution was drawn off, and the precipitate sucked as dry as possible with a pump. After washing with alcohol and ether the preparation formed a pure white dusty powder. By this method of preparation the vignin was separated from the more soluble globulins, albumins, and proteose and from all of the other water-soluble constituents of the seed.

Glycinin.—The soy-beans were coarsely ground in the laboratory, freed from the greater part of the outer seed coats by a current of air, and the meal then extracted with petroleum benzine until most of the oil was removed. After freeing from adhering benzine by exposure to the air the coarse meal was ground fine and extracted with about three parts of 10 per cent NaCl solution. Owing to the very viscid character of the extract it was necessary to mix with the meal and solvent enough dry finely divided filter paper to make it possible to squeeze out the extract in a powerful screw press. The extract thus obtained was filtered perfectly clear and dialyzed for 4 days in running water. The globulin that separated was filtered out; dissolved again in NaCl solution, and again precipitated by dialysis. After repeating this process a third time the precipitated glycinin was washed thoroughly with water, absolute alcohol, and ether and then dried over H_2SO_4. By this repeated precipitation the glycinin was separated from the more soluble proteins of the seed and had the properties and ultimate composition of preparations formerly obtained by extensive fractionation from NaCl solution.

Castor-bean globulin.—Two preparations were used for our anaphylaxis experiments. That used for experiments 1–5 was obtained by removing the shells from carefully selected castor beans and extracting the nearly oil-free meal with 10 per cent NaCl solution. The extract was filtered perfectly clear and dialyzed for 60 hours in running water. The precipitate was dissolved in 10 per cent NaCl solution and the solution saturated with NaCl. The precipitate produced was filtered out, dissolved in a dilute solution of this salt, and the solution saturated with NaCl. The second precipitate so obtained was filtered out and again subjected to the same treatment. The third precipitate produced by saturating with NaCl was dissolved in dilute NaCl solution and the clear solution dialyzed. The globulin which separated was washed with water and alcohol, dehydrated with absolute alcohol, and dried over H_2SO_4.

The preparation used for experiments 6–39 was extracted from the oil-free meal of carefully selected castor beans from which the oil had been mostly extracted with ether but from which the shells had not been removed. This meal was extracted with five times its weight of 10 per cent NaCl solution, the extract filtered perfectly clear and dialyzed for 4 days in running water. The precipitate which separated was washed with water, digested with absolute alcohol, and dried over H_2SO_4. Although this preparation was not further purified by reprecipitating, the results obtained with it agreed with those yielded by the more carefully purified preparation used for the first five experiments. The single precipitation by dialysis appears to have been sufficient to separate this globulin from the albumin (ricin) a small fraction of a milligram of which promptly kills a guinea-pig when subcutaneously injected.* From this it is probable that this crude preparation of the globulin was nearly if not wholly freed by a single precipitation by dialysis from the water-soluble proteins of the seed.

Edestin.—The hemp seeds used for making this preparation of edestin were carefully selected one by one, taking every precaution to avoid the presence of any other seeds. The seeds were then crushed under kerosene and freed from most of the oil by pressing with a hydraulic press and from the adherent kerosene by exposing to the air. The coarse meal was then ground and sifted in such a way, on sieves of suitable mesh, as to remove most of the outer coat of the seed. The meal was then extracted with a 10 per cent solution of NaCl. The extract was filtered perfectly clear and dialyzed for 4 days in running water. The crystalline precipitate thus obtained was washed with 50 per cent alcohol until the washings were free from chlorin, dehydrated by digesting with absolute alcohol, and then extracted with ether. The preparation was then dried in the air. In making this preparation apparatus which had never been used in the preparation of any vegetable proteins was used throughout, and the preparations may be regarded as entirely free from any substance others than those which it may have acquired from the hemp seeds themselves or from the water and reagents used in its preparation.

Squash-seed globulin.—Carefully selected fresh seeds of the squash were coarsely ground without removing the outer seed coats, and then extracted with benzine until most of the oil had been removed. By sifting the coarse meal on sieves of suitable mesh the greater part of the outer coats of the seeds was removed. The fine meal was then extracted with 10 per cent NaCl solution, the extract filtered perfectly clear and precipitated by dialysis. The crystalline precipitate was redissolved in 10 per cent NaCl solution, the solution filtered clear and reprecipitated by dialysis. The precipitate was washed with water, dehydrated with absolute alcohol, and dried over H_2SO_4.

Squash-seed globulan.—This preparation was obtained in a similar way to that employed in making the preparation of the squash-seed globulin and represents the part of the first dialysis precipitate which failed to dissolve in NaCl solution. It is, therefore, that part of the globulin of the squash seed which had been converted into the insoluble condition during the first dialysis.

Flax-seed globulin.—This was extracted from finely ground flax seed by a 10 per cent solution of $BaCl_2$. The extract was filtered perfectly clear and dialyzed in running water until the globulin was nearly all precipitated in octahedral crystals. The dialysis precipitate was washed with water, redissolved in NaCl solution and reprecipitated by dialysis. The crystalline separation was washed with water and alcohol and dried over H_2SO_4.

Cocoanut proteins.—The preparations of these proteins were made in the Sheffield Laboratory of Physiological Chemistry of Yale University and kindly presented to us by Professor Lafayette B. Mendel. The details of the method by which they were prepared are unknown by us.

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* Osborne, Mendel, and Harris, *Am. Jour. of Physiol.*, 1905, 14, p. 278.

Excelsin.—The oil-free meal of the Brazil nut was extracted with 3 per cent $(NH_4)_2SO_4$ solution heated to 50° and the perfectly clear extract dialyzed until the greater part of the dissolved excelsin was deposited as crystals. These were then washed thoroughly with dilute NaCl solution and then with dilute alcohol which was gradually increased in strength up to absolute alcohol. The preparation was then dried over H_2SO_4. The product thus obtained consisted wholly of hexagonal crystals and under the microscope appeared to be entirely free from any admixture with other substances.

Gliadin, wheat.—The preparation of gliadin used in these experiments was made from a high grade of wheat flour from which the gluten was obtained by washing with water. The gluten was coarsely divided by passing through a meat chopper and extracted with 70 per cent alcohol until practically all of the protein that was soluble therein had been removed. The extract was filtered perfectly clear and concentrated at a low temperature, care being taken to add from time to time a quantity of strong alcohol sufficient to prevent the gliadin separating from the solution. The concentrated extract was then precipitated by pouring into several volumes of water containing a little NaCl. The precipitate was then redissolved in 70 per cent alcohol and precipitated by pouring into a large volume of 95 per cent alcohol. The gliadin was then dehydrated with absolute alcohol, and digested twice with a large quantity of ether. After drying over H_2SO_4 it was ground to a fine white powder. During the preparation of this gliadin care was taken to avoid exposing its solutions to a temperature above 60° or 70°. This preparation was made with new apparatus and with special precaution to avoid its contamination with any other substances than those present in the seeds from which the flour was obtained or the reagents used in making it.

Gliadin, rye.—The rye flour used for this preparation was ground in the laboratory from a quantity of selected seeds which were presumably free from those of other species. Whether or not the seeds of wheat were entirely absent cannot be definitely stated because, owing to the similar appearance of these two grains, the exclusion of such a contamination cannot be assured without making a careful examination of each seed before grinding, which was not done in this case, for this preparation was not originally intended for anaphylaxis experiments. There is little doubt, however, that the seeds ground contained very few, if any, grains of wheat. The flour, from which most of the bran had been removed, was extracted with cold 70 per cent alcohol and the extract filtered perfectly clear and concentrated under diminished pressure from a water-bath at 70°, care being taken during the concentration to prevent separation of the gliadin by adding absolute alcohol as soon as the solution began to foam. The concentrated solution was cooled and then poured into a large volume of ice water, to which a very little NaCl was added in order to cause the gliadin to separate in a flocculent condition. The precipitate which settled after standing for some hours formed a coherent deposit from which the solution was poured off nearly completely. This deposit was then dissolved in a little warm alcohol and the cold concentrated solution again poured into much water as before. The gliadin was then dissolved in alcohol and poured into much absolute alcohol which gave a milky colloidal solution which immediately yielded a voluminous flocculent precipitate on adding a few drops of saturated NaCl solution. This precipitate soon settled to a coherent mass from which the alcohol was nearly completely poured off. This mass was then repeatedly digested with portions of absolute alcohol until it could be rubbed down to a fine powder. This was then washed with ether and dried over H_2SO_4.

Hordein.—A high grade of very white barley flour was used in making this preparation. This was extracted with warm 70 per cent alcohol, the extract filtered perfectly clear and concentrated to a small volume under low pressure on a water bath. On cooling, nearly all of the hordein separated as a coherent mass which was washed with water, dissolved in warm 70 per cent alcohol, and its clear solution poured into several volumes of ice water. The hordein was then again dissolved in a small quantity of 70 per cent alcohol and precipitated by pouring into much absolute alcohol. After digesting the hordein with absolute alcohol and ether it was dried over H_2SO_4 and obtained as a snow-white powder.

Zein.—This preparation was made from finely ground yellow maize meal by extracting with warm 92 per cent alcohol. The extract was filtered perfectly clear, concentrated under diminished pressure, and precipitated by pouring into a large volume of cold water containing a very little NaCl. The precipitate was then dissolved in warm 92 per cent alcohol and again precipitated as before by pouring into water. After redissolving in a small quantity of warm 92 per cent alcohol the clear solution was poured into a large volume of absolute alcohol and the precipitate digested with absolute alcohol and ether and then dried over H_2SO_4.

6. METHOD OF CONDUCTING EXPERIMENTS AND DESCRIBING RESULTS.

The experiments were performed with healthy young guinea-pigs, nearly all from one stock which was known to give strong anaphylaxis reactions. (There are some strains of guinea-pigs, as reported by Vasconcellos[53], and as we also have observed, which will not give satisfactory reactions.) In most instances the weight was about 300 gms. at the time of the first injection, some being a little smaller but none as heavy as 500 gms. The animals were fed, unless otherwise specified, exclusively upon oats, carrots, and hay. The vegetable proteins were generally dissolved in 0.2 per cent NaOH and the solution diluted with an equal volume of water before injection, making a 0.1 per cent NaOH solution, which ordinarily caused no symptoms of itself, although occasionally certain protein solutions caused an immediate transient irritation, the animals jumping about madly for a moment, but quieting down in a minute or two so that the anaphylaxis reaction was not obscured. All injections were made into the peritoneal cavity, with a blunt needle to avoid perforation of the viscera. Because of the tendency of vegetable proteins to cause agglutination of erythrocytes, and because our proteins were in alkaline solution, no intravascular injections were made.

To avoid contamination of the injected proteins with foreign proteins special precautions were adopted, necessary because of the extremely small dose of protein which may sensitize an animal. Only all-glass syringes were used, and after each set of injections they were thoroughly washed and then left standing over night in a cleaning fluid consisting of concentrated sulphuric acid and potassium dichromate. All scale pans, mortars, and other glass utensils with which the proteins came in contact were cleaned in the same manner. The needles were washed first with water and then with alcohol, and were kept constantly in absolute alcohol.

In recording the effects produced after the usual incubation period by the second or intoxicating dose, certain terms are used with the following constant significance.

1. "Doubtful" symptoms. The animal scratches itself a few times and perhaps seems a little uneasy or a trifle ill; the temperature remains normal or falls not more than 1° to 1.5° C. Such results may be occasionally observed after injection of foreign proteins into non-sensitized animals, and are not regarded as of any significance.

2. "Slight" symptoms. The animal does not become seriously ill, but scratches itself vigorously, is either very restless or very somnolent or both alternately, hair roughened, eyes usually lachrymate and partly closed. The temperature usually falls 1° to 1.5° C. Such a reaction is believed to indicate usually, if not always, a real sensitization and a true anaphylactic intoxication, but it may possibly sometimes, but rarely, occur from foreign proteins without sensitization.

3. "Moderate" symptoms. More seriously ill than under 2. Usually the hair becomes very rough, with respiratory distress and marked lachrymation, fall of temperature of 1.5°–3.5° C.; frequently the animal lies down and sometimes there is violent coughing.

4. "Severe" symptoms. Same as under 3 but so much more severe as to threaten to cause death, but followed by complete recovery in a few hours. In these animals the temperature usually falls 3° to 4°, or even more.

The results of our experiments are given in the following tables:

7. TABLES OF RESULTS OBTAINED.

TABLE 1.

CASTOR-BEAN GLOBULIN (*Ricinus communis*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Castor-bean Globulin		Castor-bean Globulin		
1. 0.0 (Control)...	..	0.1	none	
2. 0.0 (Control)...	..	0.1	doubtful	
3. 0.0 (Control)...	..	0.1	none	
4. 0.002..........	18	0.1	died in 1 hr.	
5. 0.0002.........	18	0.1	" " 6 hrs.	
6. 0.002..........	21	0.1	moderate	
7. 0.0002.........	21	0.1	died in 1 hr.	
		Flaxseed Globulin		
8. 0.002..........	18	0.1	slight	died 24 hrs. later
9. 0.0002.........	18	0.1	"	" 36 " "
10. 0.0001.........	18	0.1	doubtful	48 hrs. later reacted slightly to castor-bean globulin
11. 0.00002........	18	0.1	"	48 hrs. later reacted severely to castor-bean globulin.
12. 0.002..........	27	0.1	slight	4 days later reacted severely to castor-bean globulin
13. 0.001..........	27	0.1	none	4 days later reacted fatally to castor-bean globulin
14. 0.0002.........	27	0.1	died in 1 hr.	
15. 0.002..........	27	0.1	slight	4 days later reacted severely to castor-bean globulin
16. 0.001..........	27	0.1	"	4 days later reacted fatally to castor-bean globulin
17. 0.0002.........	27	0.1	died in 35 min.	
		Squash-seed Globulin		
18. 0.002..........	21	0.1	doubtful	3 days later reacted moderately to castor-bean globulin
19. 0.001..........	21	0.1	"	3 days later reacted fatally to castor-bean globulin
20. 0.0002.........	21	0.1	"	3 days later reacted moderately to castor-bean globulin
21. 0.0001.........	21	0.1	"	3 days later reacted fatally to castor-bean globulin
		Edestin		
22. 0.007..........	17	0.1	none	
23. 0.002..........	17	0.1	doubtful	48 hrs. later reacted severely to castor-bean globulin
24. 0.001..........	17	0.1	none	
25. 0.0002.........	17	0.1	"	
		Excelsin		
26. 0.01...........	17	0.1	none	
27. 0.001..........	17	0.1	"	
28. 0.0002.........	17	0.1	"	48 hrs. later reacted severely to castor-bean globulin
Zein		Castor-bean Globulin		
29. 0.001..........	20	0.1	none	
Squash-seed Globulin				
30. 0.01...........	22	0.1	"	
31. 0.002..........	22	0.1	"	24 hrs. later reacted fatally to squash-seed globulin
32. 0.0002.........	22	0.1	"	24 hrs. later reacted fatally to squash-seed globulin
Edestin				
33. 0.01...........	22	0.1	"	
34. 0.002..........	22	0.1	"	24 hrs. later reacted fatally to edestin
35. 0.001..........	22	0.1	"	
36. 0.0002.........	22	0.1	severe	24 hrs. later reacted severely to edestin
Excelsin				
37. 0.002..........	21	0.1	none	
38. 0.001..........	21	0.1	"	
39. 0.0002.........	21	0.1	"	48 hrs. later reacted fatally to excelsin

TABLE 1.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Flaxseed Globulin		Castor-bean Globulin		
40. 0.01	20	0.1	slight	48 hrs. later reacted moderately to flax-seed globulin
41. 0.01	20	0.1	"	48 hrs. later reacted moderately to flax-seed globulin
42. 0.002	20	0.1	"	48 hrs. later reacted moderately to flax-seed globulin
43. 0.0002	20	0.1	"	48 hrs. later reacted moderately to flax-seed globulin

TABLE 2.

FLAX-SEED GLOBULIN (*Linum usitatissimum*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
		Flax-seed Globulin		
1. 0.0 (Control)...	..	0.1	none	Temperature fell 1.1° C.
2. 0.0 (Control)...	..	0.1	"	" " 1.6° C.
Castor-bean Globulin				
3. 0.002	27	0.1	slight	4 days later reacted severely to castor-bean globulin
4. 0.001	27	0.1	none	4 days later reacted fatally to castor-bean globulin
5. 0.0002	27	0.1	died in 1 hr.	
6. 0.002	27	0.1	slight	4 days later reacted severely to castor-bean globulin
7. 0.001	27	0.1	doubtful	4 days later reacted fatally to castor-bean globulin
8. 0.002	18	0.1	slight	died 24 hrs. later
9. 0.0002	18	0.1	"	" 36 " "
10. 0.0001	18	0.1	doubtful	48 hrs. later reacted slightly to castor-bean globulin
11. 0.00002	18	0.1	"	48 hrs. later reacted severely to castor-bean globulin
12. 0.0002	27	0.1	died in 35 min.	
Squash-seed Globulin				
13. 0.008	20	0.1	none	3 days later reacted fatally to squash-seed globulin
14. 0.001	20	0.1	"	
15. 0.0001	20	0.1	"	3 days later reacted fatally to squash-seed globulin
Flax-seed Globulin		Squash-seed Globulin		
16. 0.002	20	0.1	none	24 hrs. reacted severely to flax-seed globulin
17. 0.001	20	0.1	slight	24 hrs. reacted severely to flax-seed globulin
18. 0.001	20	0.1	doubtful	24 hrs. reacted fatally to flax-seed globulin
19. 0.0002	20	0.1	none	24 hrs. reacted fatally to flax-seed globulin
		Castor-bean Globulin		
20. 0.01	20	0.1	slight	24 hrs. reacted moderately to flax-seed globulin
21. 0.01	20	0.1	"	24 hrs. reacted moderately to flax-seed globulin
22. 0.002	20	0.1	"	24 hrs. reacted moderately to flax-seed globulin
23. 0.0002	20	0.1	moderate	24 hrs. reacted moderately to flax-seed globulin

TABLE 3.

SQUASH-SEED GLOBULIN (*Cucurbita maxima*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Squash-seed Globulin		Squash-seed Globulin		
1. 0.0 (Control)	..	0.1	doubtful	
2. 0.0 (Control)	..	0.1	“	
3. 0.0 (Control)	..	0.1	“	
4. 0.01	23	0.1	died in 75 min.	
5. 0.008	23	0.1	“ “ 45 “	
6. 0.001	23	0.1	slight	
7. 0.001	23	0.1	died in 50 min.	
8. 0.0002	22	0.05	“ “ 45 “	
		Excelsin		
9. 0.01	20	0.1	moderate	3 days later reacted fatally to squash-seed globulin
10. 0.002	20	0.1	died in 2 hrs.	
11. 0.001	20	0.1	moderate	3 days later reacted slightly to squash-seed globulin
12. 0.01	21	0.1	“	24 hrs. later reacted slightly to squash-seed globulin
13. 0.002	21	0.1	“	
14. 0.001	21	0.1	“	
15. 0.0002	21	0.1	“	
16. 0.01	18	0.1	doubtful	48 hrs. later reacted moderately to squash-seed globulin
17. 0.002	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
18. 0.001	18	0.1	doubtful	48 hrs. later reacted fatally to squash-seed globulin
19. 0.0002	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
		Flax-seed Globulin		
20. 0.008	20	0.1	none	3 days later reacted fatally to squash-seed globulin
21. 0.001	20	0.1	“	
22. 0.0001	20	0.1	“	3 days later reacted fatally to squash-seed globulin
		Edestin		
23. 0.01	21	0.1	slight	
24. 0.002	21	0.1	“	
25. 0.001	21	0.1	“	
26. 0.0002	21	0.1	“	24 hrs. later reacted fatally to squash-seed globulin
27. 0.01	18	0.1	none	48 hrs. later reacted fatally to squash-seed globulin
28. 0.002	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
29. 0.001	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
30. 0.0002	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
		Castor-bean Globulin		
31. 0.01	22	0.1	none	
32. 0.002	22	0.1	“	24 hrs. later reacted fatally to squash-seed globulin
33. 0.0002	22	0.1	“	24 hrs. later reacted fatally to squash-seed globulin
Excelsin		Squash-seed Globulin		
34. 0.01	21	0.1	none	6 days later reacted fatally to excelsin
35. 0.001	21	0.1	doubtful	6 days later reacted fatally to excelsin
36. 0.0002	21	0.1	none	6 days later reacted severely to excelsin

TABLE 3.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Castor-bean Globulin		Squash-seed Globulin		
37. 0.002..........	21	0.1	doubtful	3 days later reacted moderately to castor-bean globulin
38. 0.001..........	21	0.1	“	3 days later reacted fatally to castor-bean globulin
39. 0.0002.........	21	0.1	“	3 days later reacted moderately to castor-bean globulin
40. 0.0001.........	21	0.1	“	3 days later reacted fatally to castor-bean globulin
Edestin				
41. 0.01...........	21	0.1	doubtful	24 hrs. later reacted slightly to edestin
42. 0.002..........	21	0.1	none	24 hrs. later reacted moderately to edestin
43. 0.001..........	21	0.1	“	24 hrs. later reacted doubtfully to edestin
44. 0.0002.........	21	0.1	“	24 hrs. later reacted moderately to edestin
Flax-seed Globulin				
45. 0.002..........	20	0.1	none	24 hrs. reacted severely to flax-seed globulin
46. 0.001..........	20	0.1	slight	24 hrs. reacted severely to flax-seed globulin
47. 0.001..........	20	0.1	doubtful	24 hrs. reacted fatally to flax-seed globulin
48. 0.0002.........	20	0.1	none	24 hrs. reacted fatally to flax-seed globulin
Squash-seed Globulan				The following six experiments with globulan
49. 0.02...........	15	0.125	slight	
50. 0.002..........	15	0.125	severe	
51. 0.0002.........	15	0.125	died in 90 min.	
52. 0.1............	100	0.125	slight	
53. young of 52, 6 weeks old......		0.1	“	Inheritance test
54. Young of 52, 6 weeks old......		0.1	slight	Inheritance test
Squash-seed Globulin		Squash-seed Globulin		
55. 0.1	17	0.1	severe	Died after 6 hours
56. 0.2............	17	0.1	“	“ “ “ “
57. 0.3............	17	0.1	“	Recovered
58. 0.00005........	18	0.1	died in 30 min.	
59. 0.000001.......	18	0.1	“ “ “ “	
60. 0.0000005......	18	0.1	moderate	Temp. fell 3.3°
61. 0.0000005......	18	0.1	died in 60 min.	
62. 0.0000001......	18	0.1	none	No fall in temp.
63. 0.0000001......	18	0.1	doubtful	“ “ “ “
64. 0.00000001.....	18	0.1	none	
65. 0.00000001.....	18	0.1	none	
66. 0.0005.........	21	0.020	moderate	Died during night
67. 0.0005.........	21	0.010	severe	Died after 6 hours
68. 0.0005.........	21	0.005	moderate	Temp fell 2.7°
69. 0.0005.........	21	0.005	“	“ “ 2.7°
70. 0.0005.........	21	0.004	“	“ “ 3.3°
71. 0.0005.........	21	0.003	slight	“ “ 1.6°
72. 0.0005.........	21	0.001	doubtful	“ “ 1.6°
73. 0.0005.........	21	0.001	slight	“ “ 2.7°
74. 0.0005.........	21	0.0005	none	No fall in temp.
75. 0.0005.........	21	0.0001	“	“ “ “ “
76. 0.0005.........	6	0.1	“	“ “ “ “
77. 0.0005.........	7	0..1	“	“ “ “ “
78. 0.0005.........	8	0.1	moderate	Temp. fell 3.3°
79. 0.0005.........	9	0.1	died in 60 min.	

TABLE 4.

EDESTIN (*Cannabis sativa*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Edestin		Edestin		
1. 0.0 (Control) ...	..	0.1	none	No fall in temperature
2. 0.1	18	0.25	doubtful	Animal used before with animal proteins
3. 0.02	18	0.15	moderate	Animal used before with animal proteins
4. 0.002	18	0.15	"	Animal used before with animal proteins
5. 0.002	18	0.25	severe	Animal used before with animal proteins
6. 0.0002	18	0.15	slight	Animal used before with animal proteins
7. 0.002	21	0.1	moderate	Animal used before with animal proteins
8. 0.01	19	0.1	"	24 hrs. later reacted negatively to edestin
9. 0.00005	19	0.1	slight	
10. 0.00001	19	0.1	moderate	
11. 0.000005	19	0.1	"	
12. 0.000001	19	0.1	slight	
13. 0.0000005	20	0.1	moderate	
14. 0.0000001	20	0.1	"	
15. 0.0005	19	0.1	died in 2 hrs.	
16. 0.0005	19	0.04	severe	
17. 0.0005	18	0.02	"	Temp. fell 5°. 48 hrs. later no reaction to edestin
18. 0.0005	17	0.01	moderate	Temp. fell 2.5°
19. 0.0005	17	0.005	slight	Temp. fell 2.6°. 48 hrs. later moderate reaction to edestin
20. 0.0005	18	0.002	"	Temp. fell 1.6°. 48 hrs. later no reaction to edestin
21. 0.0005	19	0.001	doubtful	48 hrs. later no reaction to edestin
		Castor-bean Globulin		
22. 0.01	22	0.1	none	
23. 0.002	22	0.1	"	24 hrs. later reacted fatally to edestin
24. 0.001	22	0.1	"	
25. 0.0002	22	0.1	severe	24 hrs. later reacted severely to edestin
		Excelsin		
26. 0.01	21	0.1	slight	24 hrs. later reacted negatively to edestin
27. 0.002	21	0.1	moderate	24 hrs. later reacted moderately to edestin
28. 0.001	21	0.1	"	24 hrs. later reacted fatally to edestin
29. 0.0002	21	0.1	"	24 hrs. later reacted moderately to edestin
30. 0.01	18	0.1	none	48 hrs. later reacted doubtfully to edestin
31. 0.002	18	0.1	"	48 hrs. later reacted doubtfully to edestin
32. 0.001	18	0.1	"	48 hrs. later reacted doubtfully to edestin
33. 0.0002	18	0.1	"	48 hrs. later reacted doubtfully to edestin
		Squash-seed Globulin		
34. 0.01	21	0.1	doubtful	24 hrs. later reacted slightly to edestin
35. 0.002	21	0.1	none	24 hrs. later reacted moderately to edestin
36. 0.001	21	0.1	"	24 hrs. reacted doubtfully to edestin
37. 0.0002	21	0.1	"	24 hrs. later reacted moderately to edestin

TABLE 4.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Castor-bean Globulin		Edestin		
38. 0.007..........	17	0.1	none	
39. 0.002..........	17	0.1	doubtful	48 hrs. later reacted severely to castor-bean globulin
40. 0.001..........	17	0.1	none	
41. 0.0002.........	17	0.1	“	
Excelsin				
42. 0.01...........	21	0.1	doubtful	
43. 0.002..........	21	0.1	“	48 hrs. later reacted severely to excelsin
44. 0.001..........	21	0.1	“	
45. 0.0002.........	21	0.1	“	
Squash-seed Globulin				
46. 0.01...........	21	0.1	slight	
47. 0.002..........	21	0.1	“	
48. 0.001..........	21	0.1	“	
49. 0.0002.........	21	0.1	“	24 hrs. later reacted fatally to squash-seed globulin
50. 0.01...........	18	0.1	none	48 hrs. later reacted fatally to squash-seed globulin
51. 0.002..........	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
52. 0.001..........	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin
53. 0.0002.........	18	0.1	“	48 hrs. later reacted fatally to squash-seed globulin

TABLE 5.

EXCELSIN (*Bertholletia excelsa*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Excelsin		Excelsin		
1. 0.0 (Control)...	..	0.1	slight	Temperature fell 1.6°
2. 0.0 (Control)...	..	0.1	doubtful	No fall in temperature
3. 0.0 (Control)...	..	0.1	none	“ “ “ “
4. 0.0 (Control)...	..	0.1	“	Temperature fell 1°
5. 0.0 (Control)...	..	0.1	“	“ “ 1.5°
6. 0.02...........	17	0.125	moderate	Animal previously used with animal proteins
7. 0.01...........	17	0.125	died in 40 min.	Animal previously used with animal proteins
8. 0.002..........	17	0.125	“ “ 120 “	Animal previously used with animal proteins
9. 0.0002.........	17	0.125	“ “ 90 “	Animal previously used with animal proteins
10. 0.002..........	19	0.1	“ “ 40 “	
11. 0.001..........	19	0.1	“ “ 45 “	
12. 0.0002.........	19	0.1	“ “ 30 “	
		Vignin		
13. 0.1............	17	0.12	none	24 hrs. later reacted severely to excelsin
14. 0.02...........	17	0.1	“	24 hrs. later reacted severely to excelsin
		Glycinin		
15. 0.002..........	16	0.1	doubtful	3 days later reacted fatally to excelsin
16. 0.001..........	16	0.1	none	3 days later reacted fatally to excelsin
17. 0.0002.........	16	0.1	“	3 days later reacted fatally to excelsin

TABLE 5.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Excelsin		Squash-seed Globulin		
18. 0.01	21	0.1	none	6 days later reacted fatally to excelsin
19. 0.001	21	0.1	doubtful	6 days later reacted fatally to excelsin
20. 0.0002	21	0.1	none	6 days later reacted severely to excelsin
		Edestin		
21. 0.01	21	0.1	doubtful	
22. 0.002	21	0.1	"	48 hrs. later reacted severely to excelsin
23. 0.001	21	0.1	"	
24. 0.0002	21	0.1	"	
		Castor-bean Globulin		
25. 0.002	21	0.1	none	
26. 0.001	21	0.1	"	
27. 0.0002	21	0.1	"	48 hrs. later reacted fatally to excelsin
Glycinin		Excelsin		
28. 0.01	22	0.1	"	48 hrs. later reacted severely to glycinin
29. 0.002	22	0.1	"	48 hrs. later reacted severely to glycinin
30. 0.001	16	0.1	doubtful	3 days later reacted severely to glycinin
31. 0.0002	16	0.1	none	3 days later reacted severely to glycinin
Squash-seed Globulin				
32. 0.01	20	0.1	moderate	3 days later reacted fatally to squash-seed globulin
33. 0.002	20	0.1	died in 2 hrs.	
34. 0.001	20	0.1	moderate	3 days later reacted slightly to squash-seed globulin
35. 0.01	21	0.1	"	24 hrs. later reacted fatally to squash-seed globulin
36. 0.002	21	0.1	"	
37. 0.001	21	0.1	"	
38. 0.0002	21	0.1	"	
39. 0.01	18	0.1	doubtful	48 hrs. later reacted moderately to squash-seed globulin
40. 0.002	18	0.1	"	48 hrs. later reacted fatally to squash-seed globulin
41. 0.001	18	0.1	"	48 hrs. later reacted fatally to squash-seed globulin
42. 0.0002	18	0.1	"	48 hrs. later reacted fatally to squash-seed globulin
Castor-bean Globulin				
43. 0.01	17	0.1	none	
44. 0.001	17	0.1	"	
45. 0.0002	17	0.1	"	48 hrs. later reacted severely to castor-bean globulin
Edestin				
46. 0.01	21	0.1	doubtful	24 hrs. later reacted negatively to edestin
47. 0.002	21	0.1	moderate	24 hrs. later reacted moderately to edestin
48. 0.001	21	0.1	"	24 hrs. later reacted fatally to edestin
49. 0.0002	21	0.1	"	24 hrs. later reacted moderately to edestin
50. 0.01	18	0.1	none	48 hrs. later reacted doubtfully to edestin
51. 0.002	18	0.1	"	48 hrs. later reacted doubtfully to edestin
52. 0.001	18	0.1	"	48 hrs. later reacted doubtfully to edestin
53. 0.0002	18	0.1	"	48 hrs. later reacted doubtfully to edestin

TABLE 6.

COCOANUT PROTEINS (*Cocos nucifera*).

	Sensitizing Dose	Days' Interval	Second Injection	Symptoms
1. 0.1	Cocoanut Protein F	19	0.15 Cocoanut Protein F	severe
2. 0.002	“ “ “	19	0.15 “ “ “	“
3. 0.01	“ “ “	19	0.15 “ “ “	“
4. 0.01	“ “ “	17	0.15 “ “ “	moderate
5. 0.01	“ “ “	17	0.16 “ Globulin B	slight
6. 0.025	“ Globulin B	24	0.16 “ Protein F	moderate
7. 0.005	“ “ “	17	0.1 “ Globulin B	severe
8. 0.005	“ “ “	17	0.1 “ “ “	“

TABLE 7.

LEGUMIN, PEA (*Pisum sativum*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Pea Legumin		Pea Legumin		
1. 0.0 (Control)	..	0.1	none	
2. 0.0 (Control)	..	0.1	“	No fall in temperature
3. 0.02	22	0.2	moderate	Had previously been used with animal protein
4. 0.002	22	0.2	“	Had previously been used with animal protein
5. 0.0002	22	0.2	slight	Had previously been used with animal protein
6. 0.00002	22	0.2	doubtful	Had previously been used with animal protein
7. 0.05	14	0.125	moderate	
8. 0.02	14	0.125	“	3 days later no reaction to pea legumin
9. 0.01	17	0.125	“	
10. 0.01	14	0.125	“	
		Vetch Legumin		
11. 0.01	14	0.125	doubtful	3 days later reacted moderately to pea legumin
12. 0.01	19	0.1	severe	
13. 0.002	19	0.1	died in 40 min.	
14. 0.001	19	0.1	“ “ 45 “	
15. 0.0005	19	0.1	“ “ 60 “	
16. 0.01	18	0.1	moderate	48 hrs. later reacted doubtfully to pea legumin
17. 0.002	18	0.1	“	48 hrs. later reacted slightly to pea legumin
18. 0.001	18	0.1	moderate	48 hrs. later reacted negatively to pea legumin
19. 0.0002	18	0.1	doubtful	died 36 hours later
		Vicilin		
20. 0.01	20	0.1	died in 20 min.	
21. 0.002	20	0.1	“ “ 35 “	
22. 0.001	20	0.1	“ “ 40 “	
23. 0.0002	20	0.1	“ “ 90 “	
		Vignin		
24. 0.01	20	0.1	doubtful	4 days later reacted severely to pea legumin
25. 0.002	20	0.1	“	4 days later reacted fatally to pea legumin
26. 0.001	20	0.1	“	4 days later reacted severely to pea legumin
27. 0.0002	20	0.1	“	4 days later reacted fatally to pea legumin
28. 0.01	22	0.1	“	24 hrs. later reacted fatally to pea legumin
29. 0.002	22	0.1	“	
30. 0.001	22	0.1	“	
31. 0.0002	22	0.1	“	24 hrs. later reacted severely to pea legumin

TABLE 7.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vetch Legumin		Pea Legumin		
32. 0.02	17	0.125	slight	24 hrs. later reacted moderately to vetch legumin
33. 0.01	14	0.1	moderate	3 days later reacted slightly to vetch legumin
34. 0.01	18	0.1	slight	24 hrs. later reacted slightly to vetch legumin
35. 0.002	18	0.1	moderate	24 hrs. later reacted slightly to vetch legumin
36. 0.001	18	0.1	slight	24 hrs. later reacted severely to vetch legumin
37. 0.0002	18	0.1	moderate	24 hrs. later reacted fatally to vetch legumin
38. 0.01	18	0.1	doubtful	48 hrs. later reacted doubtfully to vetch legumin
39. 0.002	18	0.1	"	48 hours later reacted doubtfully to vetch legumin
40. 0.001	18	0.1	"	48 hrs. later reacted doubtfully to vetch legumin
41. 0.0002	18	0.1	"	48 hours later reacted doubtfully to vetch legumin
Vicilin				
42. 0.01	20	0.1	severe	
43. 0.002	20	0.1	died in 6 hrs.	
44. 0.001	20	0.1	" " 50 min.	
45. 0.0002	20	0.1	" " 6 hrs.	
Vignin				
46. 0.01	22	0.1	doubtful	48 hrs. later reacted severely to vignin
47. 0.002	22	0.1	"	
48. 0.001	22	0.1	"	
49. 0.0002	22	0.1	"	48 hrs. later reacted fatally to vignin

TABLE 8.

LEGUMIN, VETCH (*Vicia sativa*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vetch Legumin		Vetch Legumin		
1. 0.0 (Control)	..	0.1	none	
2. 0.0 (Control)	..	0.1	"	
3. 0.0 (Control)	..	0.1	doubtful	No fall in temperature
4. 0.0 (Control)	..	0.1	"	" " " "
5. 0.1	20	0.125	moderate	Had previously been used with animal proteins
6. 0.02	20	0.125	"	Had previously been used with animal proteins
7. 0.0002	20	0.125	died in 165 min.	Had previously been used with animal proteins
8. 0.05	20	0.125	moderate	Had previously been used with animal proteins
9. 0.01	14	0.125	severe	
10. 0.01	14	0.125	died in 150 min.	
		Glycinin		
11. 0.002	20	0.125	none	3 hrs. later reacted moderately with vetch legumin
12. 0.01	18	0.1	"	24 hrs. later reacted moderately with vetch legumin
13. 0.002	18	0.1	"	24 hrs. later reacted negatively with vetch legumin
14. 0.001	18	0.1	"	24 hrs. later reacted moderately with vetch legumin
15. 0.0002	18	0.1	"	24 hrs. later reacted negatively with vetch legumin

TABLE 8.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vetch Legumin		Pea Legumin		
16. 0.01	14	0.1	moderate	3 days later reacted slightly with vetch legumin
17. 0.01	18	0.1	slight	24 hrs. later reacted slightly with vetch legumin
18. 0.002	18	0.1	moderate	24 hrs. later reacted slightly with vetch legumin
19. 0.001	18	0.1	slight	24 hrs. later reacted severely with vetch legumin
20. 0.0002	18	0.1	moderate	24 hrs. later reacted fatally with vetch legumin
21. 0.01	18	0.1	doubtful	48 hrs. later reacted doubtfully with vetch legumin
22. 0.002	18	0.1	"	48 hrs. later reacted doubtfully with vetch legumin
23. 0.001	18	0.1	"	48 hrs. later reacted doubtfully with vetch legumin
24. 0.0002	18	0.1	"	48 hrs. later reacted doubtfully with vetch legumin
		Vicilin, Pea		
25. 0.01	19	0.1	died in 45 min.	
26. 0.002	19	0.1	" " 55 "	
27. 0.001	19	0.1	" " 9 hrs.	
28. 0.0005	19	0.1	severe	24 hrs. later reacted severely with vetch legumin
29. 0.002	17	0.1	moderate	48 hrs. later reacted severely to vetch legumin
30. 0.0004	17	0.1	"	48 hrs. later reacted moderately to vetch legumin
31. 0.0004	17	0.1	"	48 hrs. later reacted severely to vetch legumin
32. 0.00005	17	0.1	"	48 hrs. later reacted severely to vetch legumin
33. 0.00005	17	0.1	"	48 hrs. later reacted severely to vetch legumin
		Vignin		
34. 0.01	21	0.1	none	3 days later reacted moderately with vetch legumin
35. 0.002	21	0.1	slight	3 days later reacted moderately with vetch legumin
36. 0.001	21	0.1	severe	3 days later reacted moderately with vetch legumin
37. 0.0002	21	0.1	"	3 days later reacted severely with vetch legumin
38. 0.01	18	0.1	none	48 hrs. later reacted doubtfully with vetch legumin
39. 0.002	18	0.1	moderate	48 hrs. later reacted doubtfully with vetch legumin
40. 0.001	18	0.1	none	48 hrs. later reacted moderately with vetch legumin
41. 0.0002	18	0.1	"	48 hrs. later reacted moderately with vetch legumin
Vicilin		Vetch Legumin		
42. 0.005	16	0.1	severe	24 hrs. later reacted severely to vicilin
43. 0.005	16	0.1	"	24 hrs. later reacted severely to vicilin
44. 0.002	16	0.1	slight	3 days later reacted slightly to vicilin
45. 0.001	16	0.1	very severe	3 days later reacted slightly to vicilin
46. 0.0005	16	0.1	" "	3 days later reacted slightly to vicilin
47. 0.01	18	0.1	slight	48 hrs. later reacted moderately to vicilin
48. 0.002	18	0.1	none	48 hrs. later reacted fatally to vicilin
49. 0.001	18	0.1	"	48 hrs. later reacted slightly to vicilin
50. 0.0002	18	0.1	doubtful	48 hrs. later reacted moderately to vicilin

TABLE 8.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Pea Legumin		Vetch Legumin		
51. 0.01	19	0.1	severe	
52. 0.002	19	0.1	died in 40 min.	
53. 0.001	19	0.1	“ “ 45 “	
54. 0.0005	19	0.1	“ “ 60 “	
55. 0.01	18	0.1	moderate	48 hrs. later reacted doubtfully to pea legumin
56. 0.002	18	0.1	“	48 hrs. later reacted slightly to pea legumin
57. 0.001	18	0.1	“	48 hrs. later reacted negatively to pea legumin
58. 0.0002	18	0.1	doubtful	died 36 hrs. later
Vignin				
59. 0.002	20	0.1	severe	24 hrs. later reacted fatally to vignin
60. 0.0002	20	0.1	died in 4 hrs.	
61. 0.01	18	0.1	“ “ 2 “	
62. 0.002	18	0.1	none	48 hrs. later reacted fatally to vignin
63. 0.001	18	0.1	“	48 hrs. later reacted fatally to vignin
64. 0.0002	18	0.1	doubtful	48 hrs. later reacted fatally to vignin

TABLE 9.

VICILIN (*Pisum sativum*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vicilin		Vicilin		
1. 0.0 (Control)	..	0.1	slight	
2. 0.0 (Control)	..	0.1	none	
3. 0.0 (Control)	..	0.1	slight	Temperature fell 1°
4. 0.0 (Control)	..	0.1	“	“ “ 1.5°
5. 0.05	18	0.16	severe	Had been used before with animal proteins
6. 0.01	18	0.16	slight	Had been used before with animal proteins
7. 0.001	18	0.16	“	Had been used before with animal proteins
8. 0.0001	18	0.20	moderate	Had been used before with animal proteins
9. 0.005	16	0.125	died in 65 min.	Fresh animals
10. 0.005	16	0.125	“ “ 95 “	“ “
		Vetch Legumin		
11. 0.005	16	0.1	severe	24 hrs. later reacted severely with vicilin
12. 0.005	16	0.1	“	24 hrs. later reacted severely with vicilin
13. 0.002	16	0.1	slight	3 days later reacted slightly with vicilin
14. 0.001	16	0.1	very severe	3 days later reacted slightly with vicilin
15. 0.0005	16	0.1	“ “	3 days later reacted slightly with vicilin
16. 0.01	18	0.1	slight	48 hrs. later reacted moderately with vicilin
17. 0.002	18	0.1	none	48 hrs. later reacted fatally with vicilin
18. 0.001	18	0.1	“	48 hrs. later reacted slightly with vicilin
19. 0.0002	18	0.1	doubtful	48 hrs. later reacted moderately to vicilin

TABLE 9.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vicilin		Pea Legumin		
20. 0.01	20	0.1	severe	
21. 0.002	20	0.1	died after 6 hrs.	
22. 0.001	20	0.1	" in 50 min.	
23. 0.0002	20	0.1	" after 6 hrs.	
Vetch Legumin		Vicilin		
24. 0.01	19	0.1	died in 45 min.	
25. 0.002	19	0.1	" " 55 "	
26. 0.001	19	0.1	" " 9 hrs.	
27. 0.0005	19	0.1	severe	24 hrs. later reacted severely with vetch legumin
28. 0.002	17	0.1	moderate	48 hrs. later reacted severely to vetch legumin
29. 0.0004	17	0.1	"	48 hrs. later reacted moderately to vetch legumin
30. 0.0004	17	0.1	"	48 hrs. later reacted severely to vetch legumin
31. 0.00005	17	0.1	"	48 hrs. later reacted severely to vetch legumin
32. 0.00005	17	0.1	"	48 hrs. later reacted severely to vetch legumin
Pea Legumin				
33. 0.01	20	0.1	died in 20 min.	
34. 0.002	20	0.1	" " 35 "	
35. 0.001	20	0.1	" " 40 "	
36. 0.0002	20	0.1	" " 90 "	
Glycinin				
37. 0.01	21	0.08	doubtful	
38. 0.002	21	0.08	"	24 hrs. later reacted severely with glycinin
39. 0.001	21	0.08	"	
40. 0.0002	21	0.08	moderate	24 hrs. later reacted fatally with glycinin

TABLE 10.

Vignin (*Vigna sinensis*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vignin		Vignin		
1. 0.0 (Control)	..	0.1	doubtful	
2. 0.0 (Control)	..	0.1	none	
3. 0.01	24	0.1	severe	
4. 0.002	20	0.1	died in 60 min.	
5. 0.001	20	0.1	" " 30 "	
6. 0.0002	20	0.1	" " 50 "	
		Vetch Legumin		
7. 0.002	20	0.1	severe	24 hrs. later reacted fatally to vignin
8. 0.0002	20	0.1	died in 4 hrs.	
9. 0.01	18	0.1	" " 2 "	
10. 0.002	18	0.1	none	48 hrs. later reacted fatally to vignin
11. 0.001	18	0.1	"	48 hrs. later reacted fatally to vignin
12. 0.0002	18	0.1	doubtful	48 hrs. later reacted fatally to vignin
		Pea Legumin		
13. 0.01	22	0.1	"	48 hrs. later reacted severely to vignin
14. 0.002	22	0.1	"	
15. 0.001	22	0.1	"	
16. 0.0002	22	0.1	"	48 hrs. later reacted fatally to vignin

TABLE 10.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vignin		Glycinin		
17. 0.01	20	0.1	doubtful	24 hrs. later reacted fatally to vignin
18. 0.002	20	0.1	none	
19. 0.001	20	0.1	"	
20. 0.0002	20	0.1	"	24 hrs. later reacted fatally to vignin
21. 0.01	24	0.1	doubtful	48 hrs. later reacted fatally to vignin
Excelsin		Vignin		
22. 0.1	17	0.1	none	24 hrs. later reacted severely to excelsin
23. 0.02	17	0.1	"	24 hrs. later reacted severely to excelsin
Glycinin				
24. 0.01	20	0.1	slight	
25. 0.002	20	0.1	severe	24 hrs. later reacted severely to glycinin
26. 0.001	20	0.1	"	24 hrs. later reacted severely to glycinin
27. 0.0002	20	0.1	severe	
28. 0.01	18	0.1	moderate	72 hrs. later reacted slightly to glycinin
29. 0.002	18	0.1	slight	72 hrs. later reacted slightly to glycinin
30. 0.001	18	0.1	none	72 hrs. later reacted slightly to glycinin
31. 0.0002	18	0.1	moderate	72 hrs. later reacted slightly to glycinin
Hordein				
32. 0.02	20	0.1	none	
Vetch Legumin				
33. 0.01	21	0.1	"	3 days later reacted moderately to vetch legumin
34. 0.002	21	0.1	slight	3 days later reacted moderately to vetch legumin
35. 0.001	21	0.1	severe	3 days later reacted moderately to vetch legumin
36. 0.0002	21	0.1	severe	3 days later reacted severely to vetch legumin
37. 0.01	18	0.1	none	48 hrs. later reacted doubtfully to vetch legumin
38. 0.002	18	0.1	moderate	48 hrs. later reacted doubtfully to vetch legumin
39. 0.001	18	0.1	none	48 hrs. later reacted moderately to vetch legumin
40. 0.0002	18	0.1	"	48 hrs. later reacted moderately to vetch legumin
Pea Legumin				
41. 0.01	20	0.1	doubtful	4 days later reacted severely to pea legumin
42. 0.002	20	0.1	"	4 days later reacted fatally to pea legumin
43. 0.001	20	0.1	"	4 days later reacted severely to pea legumin
44. 0.0002	20	0.1	"	4 days later reacted fatally to pea legumin
45. 0.01	22	0.1	"	24 hrs. later reacted fatally to pea legumin
46. 0.002	22	0.1	"	
47. 0.001	22	0.1	"	
48. 0.0002	22	0.1	"	24 hrs. later reacted severely to pea legumin
Hordein				
49. 0.006	19	0.1	doubtful	24 hrs. later reacted severely to hordein
50. 0.002	19	0.1	none	24 hrs. later reacted slightly to hordein
51. 0.001	19	0.1	"	24 hrs. later reacted severely to hordein

TABLE 11.

GLYCININ (*Soja hispida*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Glycinin		Glycinin		
1. 0.1	16	0.125	slight	Previously used with animal proteins
2. 0.02	16	0.125	"	Previously used with animal proteins
3. 0.002	16	0.125	severe	Previously used with animal proteins
4. 0.005	22	0.15	moderate	Previously used with animal proteins
5. 0.0005	22	0.15	slight	Previously used with animal proteins
6. 0.0005	22	0.15	"	Previously used with animal proteins
7. 0.01	17	0.1	severe	Normal animal
8. 0.002	17	0.1	"	" "
9. 0.001	17	0.1	died in 3 hrs.	" "
10. 0.0002	17	0.1	" " 7 "	" "
11. 0.001	16	0.1	severe	3 days later reacted negatively to glycinin
		Excelsin		
12. 0.001	16	0.1	doubtful	3 days later reacted severely to glycinin
13. 0.0002	16	0.1	none	3 days later reacted severely to glycinin
14. 0.01	22	0.1	"	2 days later reacted severely to glycinin
15. 0.002	22	0.1	"	2 days later reacted severely to glycinin
		Vignin		
16. 0.01	20	0.1	slight	
17. 0.002	20	0.1	severe	24 hrs. later reacted severely to glycinin
18. 0.001	20	0.1	"	24 hrs. later reacted severely to glycinin
19. 0.0002	20	0.1	"	
20. 0.01	18	0.1	moderate	72 hrs. later reacted slightly to glycinin
21. 0.002	18	0.1	slight	72 hrs. later reacted slightly to glycinin
22. 0.001	18	0.1	none	72 hrs. later reacted slighty to glycinin
23. 0.0002	18	0.1	moderate	72 hrs. later reacted slightly to glycinin
		Vicilin		
24. 0.01	21	0.08	doubtful	
25. 0.002	21	0.08	"	24 hrs. later reacted severely to glycinin
26. 0.001	21	0.08	"	
27. 0.0002	21	0.08	moderate	24 hrs. later reacted fatally to glycinin
Vetch Legumin		Glycinin		
28. 0.01	18	0.1	none	24 hrs. later reacted moderately to vetch legumin
29. 0.002	18	0.1	"	24 hrs. later reacted negatively to vetch legumin
30. 0.002	20	0.125	"	3 hrs. later reacted moderately to vetch legumin
31. 0.001	18	0.1	"	24 hrs. later reacted negatively to vetch legumin
32. 0.0002	18	0.1	"	24 hrs. later reacted moderately to vetch legumin
Excelsin				
33. 0.002	16	0.1	doubtful	3 days later reacted fatally to excelsin
34. 0.001	16	0.1	none	3 days later reacted fatally to excelsin
35. 0.0002	16	0.1	"	3 days later reacted fatally to excelsin

TABLE 11.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Vignin		Glycinin		
36. 0.01	20	0.1	doubtful	24 hrs. later reacted fatally to vignin
37. 0.002	20	0.1	none	
38. 0.001	20	0.1	"	
39. 0.0002	20	0.1	"	24 hrs. later reacted fatally to vignin

TABLE 12.

GLIADIN, WHEAT (*Triticum vulgare*).

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Gliadin (wheat)		Gliadin (wheat)		
1. 0.0 (Control)	..	0.1	none	
2. 0.005	22	0.1	moderate	
3. 0.005	22	0.1	died in 1 hr.	
4. 0.005	25	0.1	severe	
5. 0.005	25	0.1	died in 1 hr.	
6. 0.1	24	0.1	" " 1 "	
		Gliadin (rye) (*Secale cereale*)		
7. 0.01	24	0.1	died in 20 min.	
8. 0.002	24	0.1	" " 10 "	
9. 0.001	24	0.1	severe	3 days later reacted moderately to wheat gliadin
10. 0.0002	24	0.1	severe	3 days later reacted moderately to wheat gliadin
11. 0.001	18	0.1	died in 10 min.	
12. 0.0002	18	0.1	severe	48 hrs. later reacted slightly to wheat gliadin
13. 0.0001	18	0.1	died in 12 min.	
14. 0.00002	18	0.1	moderate	48 hrs. later reacted negatively to wheat gliadin
Gliadin (rye)		Gliadin (wheat)		
15. 0.01	24	0.1	severe	3 days later reacted negatively to rye gliadin
16. 0.002	24	0.1	severe	3 days later reacted slightly to rye gliadin
17. 0.0002	24	0.1	none	3 days later reacted negatively to rye gliadin
18. 0.001	18	0.1	severe	died 24 hrs. later
19. 0.0002	18	0.1	died in 80 min.	
20. 0.0001	18	0.1	" " 80 "	
21. 0.00002	18	0.1	severe	died 36 hrs. later
Gliadin (wheat)		Zein		
22. 0.02	26	0.25	none	
23. 0.08	26	0.25	"	
24. 0.005	22	0.1	doubtful	3 days later reacted severely to wheat gliadin
25. 0.005	22	0.1	"	3 days later reacted fatally to wheat gliadin
26. 0.002	17	0.1	none	48 hrs. later reacted moderately to wheat gliadin
27. 0.001	17	0.1	"	48 hrs. later reacted moderately to wheat gliadin
28. 0.0002	17	0.1	"	48 hrs. later reacted fatally to wheat gliadin
Zein		Gliadin (wheat)		
29. 0.005	22	0.1	doubtful	
30. 0.005	22	0.1	slight	
31. 0.01	20	0.1	none	24 hrs. later reacted slightly to zein
32. 0.002	17	0.1	"	48 " " " severely " "
33. 0.001	17	0.1	"	48 " " " " " "
34. 0.0002	17	0.1	"	48 " " " " " "

TABLE 13.

HORDEIN (*Hordeum vulgare*).

Sensitizing Dose	Days' Inter-val	Second Injection	Symptoms	Subsequent Injections and Remarks
Hordein		Hordein		
1. 0.0 (Control)	..	0.1	none	
2. 0.0 (Control)	..	0.1	"	No fall in temperature
3. 0.02	23	0.1	slight	
4. 0.01	23	0.1	died in 2 hrs.	
5. 0.002	23	0.1	severe	
6. 0.006	19	0.1	"	
7. 0.002	19	0.1	"	
8. 0.001	19	0.1	slight	
		Zein		
9. 0.02	22	0.1	none	24 hrs. later reacted slightly to hordein
10. 0.01	22	0.1	"	24 hrs. later reacted fatally to hordein
11. 0.002	22	0.1	"	24 hrs. later reacted severely to hordein
		Vignin		
12. 0.006	19	0.1	doubtful	24 hrs. later reacted severely to hordein
13. 0.002	19	0.1	none	24 hrs. later reacted slightly to hordein
14. 0.001	19	0.1	"	24 hrs. later reacted severely to hordein
Zein		Hordein		
15. 0.01	15	0.1	doubtful	3 days later reacted slightly to zein
16. 0.002	15	0.1	none	3 " " " " " "
17. 0.001	15	0.1	"	3 " " " " " "
18. 0.0002	15	0.1	"	3 " " " " " "

TABLE 14.

ZEIN (*Zea mays*).

Sensitizing Dose	Days' Inter-val	Second Injection	Symptoms	Subsequent Injections and Remarks
Zein		Zein		
1. 0.02	17	0.20	died in 1 hr.	Animal not raised on corn diet
2. 0.004	17	0.25	" " 40 min.	" " " " " "
3. 0.002	18	0.20	" " 12 hrs.	" " " " " "
4. 0.0004	17	0.20	severe	" " " " " "
5. 0.0002	18	0.20	"	" " " " " "
6. 0.01	25	0.20	died in 6 hrs.	" " " " " "
7. 0.01	25	0.20	" " 6 "	" " " " " "
8. 0.005	25	0.15	severe	" " " " " "
9. 0.02	23	0.1	none	" raised on corn diet
10. 0.01	23	0.1	"	" " " " "
11. 0.002	23	0.1	"	" " " " "
12. 0.01	17	0.1	severe	" " " bread and milk
13. 0.01	17	0.1	slight	" " " " " "
14. 0.01	18	0.1	none	" " " corn meal
15. 0.002	18	0.1	"	" " " " "
16. 0.001	18	0.1	"	" " " " "
17. 0.0003	18	0.1	"	" " " " "
18. 0.005	18	0.1	moderate	" " " oats and carrots
19. 0.001	18	0.1	severe	" " " " " "
		Gliadin (wheat)		All animals below raised on diet free from corn
20. 0.01	20	0.1	none	24 hrs. later reacted slightly to zein
21. 0.002	17	0.1	"	48 " " " severely " "
22. 0.001	17	0.1	"	48 " " " " " "
23. 0.0002	17	0.1	"	48 " " " " " "
		Hordein		
24. 0.01	15	0.1	doubtful	3 days " " slightly " "
25. 0.002	15	0.1	none	3 " " " " " "

TABLE 14.—*Continued.*

Sensitizing Dose	Days' Interval	Second Injection	Symptoms	Subsequent Injections and Remarks
Zein		Hordein		
26. 0.001..........	15	0.1	none	3 days later reacted slightly to zein
27. 0.0002.........	15	0.1	"	3 " " " " " "
		Castor-bean Globulin		
28. 0.01...........	20	0.1	none	
Hordein		Zein		
29. 0.02...........	22	0.1	"	24 hrs. later reacted slightly to hordein
30. 0.01...........	22	0.1	"	24 hrs. later reacted fatally to hordein
31. 0.002..........	22	0.1	"	24 hrs. later reacted severely to hordein
Gliadin (wheat)				
32. 0.002..........	17	0.1	"	48 hrs. later reacted moderately to gliadin
33. 0.001..........	17	0.1	"	48 hrs. later reacted moderately to gliadin
34. 0.0002.........	17	0.1	"	24 hrs. later reacted fatally to gliadin
35. 0.02...........	26	0.25	"	
36. 0.08...........	26	0.25	"	
37. 0.005..........	22	0.1	doubtful	3 days later reacted severely to gliadin
38. 0.005..........	22	0.1	"	3 days later reacted fatally to gliadin

8. COMPARISON OF ANAPHYLAXIS REACTIONS OBTAINED WITH VEGETABLE AND ANIMAL PROTEINS.

a) MINIMUM SENSITIZING AND INTOXICATING DOSES.

As can be seen from these tables the vegetable proteins have been found to be suitable for anaphylaxis work, giving reactions when used in amounts comparable to those required for producing reactions with animal proteins. In general, however, it may be said that the reactions are somewhat less severe and less constant in degree than those usually observed by the commonly used animal proteins, such as egg albumin and various sera.

The only quantitative experiments reported with purified animal proteins[14] gave as the minimum fatally sensitizing dose of crystallized egg albumin one-thousandth of a milligram (0.000,001 gm.) while five one-hundred-thousandths of a milligram (0.000,000,05 gm.) sensitized animals so that they gave some reaction on a subsequent injection of egg albumin. Fatal intoxication of sensitized 300 gm. guinea-pigs was usually produced by five-tenths milligram (0.0005 gm.) of egg albumin given intraperitoneally. For comparison a series of experiments were performed with edestin from hemp seed, the results of which are given in Table 4, experiments 1–21.

These experiments show that the sensitizing power of edestin is not noticeably less than that of crystallized egg albumin, as one ten-thousandth of a milligram (0.000,000,1 gm.), the smallest sensitizing dose tried, rendered the guinea-pig sensitive to edestin. The intoxicating power of edestin is, however, much less than that of egg albumin, serum proteins, etc., for the minimum intoxicating dose of this preparation of edestin was two to five milligrams (0.002 to 0.005 gm.), while severe reactions were not obtained with less than 20 to 40 milligrams. These quantities are large when compared with the half-milligram (0.0005 gm.) dose of egg albumin which produced fatal intoxication in sensitized guinea-pigs.

Since edestin appeared to be the least toxic, anaphylactically, of all the vegetable proteins that we have tried, with the possible exception of glycinin, we determined also the minimum sensitizing and intoxicating doses of squash-seed globulin, which seemed the most active vegetable protein used by us. The results of these experiments are given in Table 3, experiments 58 to 75. It will be seen that a sensitizing dose of one two-thousandth of a milligram (0.000,000,5 gm.) sensitized guinea-pigs fatally, while one ten-thousandth of a milligram (0.000,000,1 gm.) did not render the animals sensitive; these doses are not essentially different from those found as the minimum active doses of egg albumin and edestin, indicating that the degree of activity of different proteins in the anaphylaxis reaction bears no relation to the minimum sensitizing dose. To a certain extent the same is true for the minimum intoxicating dose, as is shown by a comparison of the results obtained with edestin and with squash-seed globulin. Although the customary intoxicating dose of 0.1 gm. usually causes death in sensitized pigs when the protein is squash-seed globulin, and the same dose of edestin usually causes only moderate and rarely severe symptoms, yet it was found that the minimum intoxicating dose was practically the same with each. It required ten to twenty milligrams of squash-seed globulin to cause severe reactions, 3 to 5 milligrams causing but slight symptoms, and amounts below that producing doubtful or no reactions in sensitized pigs. The following may be proposed as a possible explanation of this unexpected result: Edestin seems to owe much of its inefficiency in the anaphylaxis reaction to its slight solubility in the body fluids, it having been shown that alkaline solutions of edestin are precipitated in the peritoneal cavity; therefore, no matter what the amount of injected edestin may be, the peritoneal fluid cannot dissolve and carry quickly to the blood more than a small quantity of this protein, not enough ordinarily to cause severe intoxication, and no more whether 40 or 200 mg. are injected. As the minimum intoxicating doses of edestin and squash-seed globulin are practically the same, the greater toxicity of the latter probably does not depend upon a difference of composition but upon its greater solubility in the body fluids, so that it is possible for a fatal does to be absorbed from the peritoneum into the blood.

b) RELATIVE ACTIVITY OF DIFFERENT VEGETABLE PROTEINS.

Of the several vegetable proteins used in these experiments, distinctly the most toxic to sensitized guinea-pigs was, as mentioned above, squash-seed globulin. This protein almost always caused fatal reactions, which were characterized by a rapid course and violent symptoms. Vignin, excelsin, and the globulin from castor bean were almost as toxic as squash-seed globulin to sensitized animals, although on the whole the reactions were not usually quite as sharp and rapid as with squash-seed globulin. Zein, vicilin, and gliadin frequently caused fatal and usually marked reactions, but these were less severe than the reactions with the proteins above named, while the legumins, hordein, and glycinin, caused fatal reactions only exceptionally. As before mentioned, edestin is distinctly inferior to the other proteins in producing anaphylactic intoxication. The following table gives some suggestion of the toxicity exhibited by the various proteins, but is not an exact indication, for in many of the experiments the intoxicating dose was given as a control to sensitized guinea-pigs that had failed to react to an injection of a heterologous protein 24 to 72 hours previously, and in such animals the reaction is commonly less severe and always less constant in degree than in animals which have not had the heterologous protein.

NUMBERS							PERCENTAGES				
Fatal	Severe	Moderate	Slight	Doubtful or Negative	Total		Fatal	Severe	Moderate	Slight	Doubtful or Negative
7	5	3	1	0	16	Castor-bean globulin.....	43	30	19	6	0
18	0	0	1	0	19	Squash-seed globulin.....	95	0	0	5	0
2	3	5	3	2	15	Edestin..................	13	20	33	20	13
11	4	1	0	0	16	Excelsin.................	69	25	6	0	0
0	5	2	1	0	8	Cocoanut proteins........	0	63	25	12	0
3	3	4	0	0	10	Legumin, pea.............	30	30	40	0	0
2	2	11	0	4	19	Legumin, vetch..........	11	11	56	0	22
3	0	2	2	0	7	Vicilin..................	44	0	28	28	0
11	4	0	0	0	15	Vignin...................	72	28	0	0	0
3	8	0	2	0	13	Glycinin.................	23	62	0	15	0
7	4	5	0	0	16	Gliadin..................	44	25	31	0	0
2	6	0	4	0	12	Hordein..................	17	50	0	34	0
5	5	1	1	0	12	Zein.....................	42	42	8	8	0

The reason or reasons for the differences in anaphylactic activity shown by these vegetable proteins we have not yet determined. One factor that may be of importance is the solubility of the several proteins in the body fluids, for we have found that there is usually more or less precipitated matter in the abdominal cavities of guinea-pigs which have received injections of proteins dissolved in 0.1 per cent NaOH. The bearing of this precipitation upon the slight toxicity of edestin has already been discussed on pp. 71 and 107.

c) SYMPTOMATOLOGY OF REACTIONS WITH VEGETABLE PROTEINS.

The observed symptoms of anaphylactic intoxication with vegetable proteins were not essentially different from those produced by animal proteins, but there were distinct quantitative differences. With the vegetable proteins the period which elapsed between the injection into a sensitized animal of the second or intoxicating dose and the appearance of symptoms was usually longer. Commonly distinct reactions did not appear in less than 20 to 30 minutes, and in fatal cases death usually occurred after 45 to 60 minutes, whereas with egg white and sera symptoms usually appeared within 15 minutes after intraperitoneal injection, frequently in 5 to 10 minutes, and death often occurred a few minutes later. As a rule the symptoms produced with the vegetable proteins were less violent, the stormy convulsive attacks so common with serum and egg albumin intoxication being seldom observed; also severe paralysis was not often produced. Sneezing, coughing, and scratching were less marked in most cases. Roughening of the hair and lachrymation were usually pronounced, and there was the typical sharp fall in temperature, but even in the fatal cases the onset and course of the symptoms were seldom violent, and the animals died more quietly. In exceptional cases, however, severe and violent symptoms with a rapidly fatal termination were produced by the vegetable proteins. In most of the fatal cases autopsy did not reveal the distended emphysematous lungs, and hemorrhages were less extensive although commonly present; that is, the conditions more closely approximated those seen in cases of serum intoxication when death has occurred slowly, without violent symptoms. The abdominal cavity usually contained considerable fluid, and masses of what seemed to be coagulated or precipitated protein. How much of this insoluble material represented precipitated vegetable proteins and how much was lymph proteins we have not investigated.

The period of incubation after injection of the sensitizing dose before the animal became sensitive was about the same as with the animal proteins, although strong reactions were not usually obtained before the fourteenth day, and for the best results it was better to wait twenty days. In a series of experiments (Table 3, Nos. 76–79) performed with squash-seed globulin for the purpose of securing definite data upon the minimum incubation period, it was found that with intraperitoneal injections no symptoms could be obtained in less than 8 days; a parallel series with egg albumin gave exactly the same result.

It has been observed with plant proteins, even more strikingly than with animal proteins, that there is commonly a less active sensitization by large doses than by small doses. If our records are gone over it will be noticed that as a usual thing animals which received a sensitizing dose of 0.01 gm. protein reacted less violently to the second dose of the same protein than animals that had received but 0.001 gm. or 0.0002 gm., as shown for example by experiments 34–36, Table 3, squash-seed globulin, and especially by experiments 55–57, in which large sensitizing doses were purposely given. Possibly this phenomenon depends upon the slow absorption of the relatively insoluble vegetable proteins, thus favoring the development of a partial immunity or defective sensitization such as is observed frequently when large amounts of serum are given for the sensitizing dose.

9. SPECIFICITY OF VEGETABLE PROTEINS AS SHOWN BY THE ANAPHYLAXIS REACTION.

a) GENERAL CONSIDERATIONS.

The vegetable proteins examined show distinct characteristics as regards specificity. In most cases sensitization with one protein rendered the animal sensitive only to the same protein but not to proteins from other more or less closely related plants. Thus castor-bean globulin (see Table 1) did not render guinea-pigs sensitive to the globulin from the squash seed, hemp seed, or Brazil nut. Occasionally atypical results were obtained, especially in the case of flax-seed globulin (experiments 8 to 17, Table 2). Of ten animals sensitized with castor-bean globulin and injected later with flax-seed globulin two died promptly with typical symptoms of anaphylaxis, two died after 24 to 36 hours, two showed slight symptoms, while the other four showed little or no reaction.

In Table 1 it is also shown that edestin, excelsin, and squash-seed globulin as well as zein did not sensitize guinea-pigs to castor-bean globulin. Here too one atypical reaction is observed (No. 36, Table 1). It is evident from these and similar irregular results that it is unsafe to draw conclusions from any small number of experiments, as some writers have incautiously done

The reason for this peculiar irregularity of reaction is not clear and no such marked irregularity was obtained with other vegetable proteins. It certainly is not due to the presence of minute quantities of contaminating flax-seed globulin in our preparation of castor-bean globulin, for otherwise reactions should have been observed with those animals which had received larger sensitizing doses than some of those which did react. As most of our preparations were not originally made for use in anaphylaxis experiments no special precautions had been taken in their handling to avoid contamination by minute traces of other proteins. To detect or avoid possible errors from this source, therefore, the sensitizing dose was purposely varied in each series from 0.01 to 0.0002 gm. and the second injection was made with but 0.1 gm. of protein, which would not contain an intoxicating dose of any admixed protein unless the impurity amounted to 1 to 10 per cent, and our extensive experience indicates that there has been no undetected error because of such impurities in the material.

As is the case with animal proteins, we have found that injection of a heterologous vegetable protein into a sensitized animal does not ordinarily render it refractory to a subsequent injection of the homologous protein as does a non-fatal reaction to the homologous protein, a point of importance in considering the specificity of the reactions. It is generally found, however, that the reaction is somewhat less severe when the homologous protein is injected 24 to 72 hours after a heterologous protein, and less often fatal than is usual with simple anaphylaxis experiments. This is probably at least partly due to incomplete absorption from the peritoneum, in consequence of the inflammatory reaction and fibrinous deposits in lymph spaces and on the peritoneal surfaces that follow the injection of the vegetable proteins. At least this factor is too evident to permit one to conclude that there has been any immunological process which reduces the reactivity of the animal. It was also noticed that animals that have been used for anaphylaxis experiments are not suitable for subsequent anaphylaxis experiments, even with very dissimilar proteins. For reasons of economy guinea-pigs that had been used in experiments with animal proteins were sometimes used shortly afterward in orientation experiments with vegetable proteins, where only gross phenomena were sought. A few only of these experiments are recorded in the accompanying tables, chiefly for comparison, and it will be seen that although reactions were obtained they were usually less severe than when fresh guinea-pigs were used (see Tables 4, 5, 7, 8, 9, 11). How much of this decrease in reactivity depends upon the factors mentioned above, and how much upon the existence and participation of non-specific anaphylactogens we have not attempted to determine.

b) SUMMARY OF RESULTS WITH COMMENTS.

The outcome of the individual experiments which are given in the tables is evident upon inspection of the data there given, but there are some features which require discussion.

Table 1. *Castor-bean globulin.*—That toxic symptoms should result from the injection of the globulin extracted from the castor bean might be expected from what we know of the great toxicity of ricin and the commonly assumed absorption of one protein by another when precipitated from solutions containing each of them. That our preparation of the castor-bean globulin did not contain a sufficient quantity of ricin to cause toxic symptoms is shown by the control experiments 1–3 and also by experiments 29–39, in only one of which were any symptoms of a toxic character observed. Our experiments with the castor-bean globulin were especially directed to a study of its anaphylactic relations to the globulins of the squash seed, flax seed, and hemp seed (edestin), because these four globulins are so nearly alike in crystalline form, solubility, physical properties, ultimate composition, and proportion of their decomposition products, that only a most critical comparison has revealed differences between them sufficient to justify the conclusion that they are distinct substances. It is therefore of interest to note that the only evidence of an anaphylaxis reaction observed between these four proteins is shown by the irregular and inconstant reactions produced in guinea-pigs sensitized by castor-bean globulin, and reinjected with flax-seed globulin, or conversely. It is possible that this difference in behavior between the flax-seed globulin and the others is caused by some difference in its structure which, if discovered, might shed much light on the nature of the anaphylaxis reaction.

Table 2. *Flax-seed globulin.*—Absolute specificity exists between this protein and the closely related globulin of squash seed, but castor-bean globulin and flax-seed globulin show a slight degree of interaction. Animals sensitized with flax-seed globulin react slightly but definitely to castor-bean globulin and are rendered partially refractory to the flax-seed globulin; but when the reverse reaction is tried and flax-seed globulin is injected into animals sensitized with castor-bean globulin, remarkably irregular reactions are

obtained, four of ten dying, five giving slight, and one no symptoms. Such irregular results are baffling, and many more experiments must be performed before any attempt can be made to interpret them.

Table 3. *Squash-seed globulin.*—Although animals sensitized with squash-seed globulin did not react to the three similar globulins from the castor bean, flax seed, or hemp seed (edestin), there was marked evidence of a reaction with excelsin, though in most cases no protection was imparted thereby. On the other hand, guinea-pigs sensitized with excelsin did not react to subsequent injections of squash-seed globulin. Excelsin differs both in crystalline form and ultimate composition to such a degree from the four globulins above mentioned, that it has long been recognized as a distinctly different protein. The structural differences between excelsin and these other globulins may however not be so great as these facts indicate, for the analyses of the products of hydrolysis of squash-seed globulin and of excelsin have given very similar results. In view of the wide difference, biologically, in the origin of these two proteins, their anaphylactic and chemical relations deserve further careful study.

Table 4. *Edestin.*—Edestin showed the least capacity to cause satisfactory anaphylactic reactions of any of the proteins tested. Compared with squash-seed globulin the difference in this respect was marked, although these two proteins appear to be, so far as known, chemically very similar to one another. On account of the relatively slight toxicity to guinea-pigs sensitized with edestin itself, and its tendency to cause slight atypical symptoms when injected into normal animals, our results were not as decisive as those obtained with the other proteins, especially when the refractory or "anti-anaphylactic" condition of the guinea-pigs was tested. Between the three similar globulins—mentioned in discussing Table 1—and edestin no reaction was obtained, but as was the case with animals sensitized with squash-seed globulin, there was also some reaction when excelsin was injected into guinea-pigs sensitized with edestin.

Table 5. *Excelsin.*—Although excelsin rendered guinea-pigs highly sensitive to a subsequent injection of excelsin, no evidence

of any reaction was obtained when such animals were treated with any of the other proteins tested, except edestin, which caused symptoms of a doubtful character. It is to be noted that while squash-seed globulin rendered guinea-pigs slightly sensitive to excelsin, this latter did not make them sensitive to squash-seed globulin. These last atypical results were obtained with one strain of guinea-pigs and not with another, and probably represent more a natural susceptibility to excelsin than a true sensitization; normal control guinea-pigs occasionally showing more or less atypical reactions when given 0.1 gm. doses of excelsin.

Table 6. *Cocoanut proteins.*—The preparations used in these experiments do not represent products of sufficiently definite character to require special comment.

Tables 7, 8, 9, 10, and 11. *Legumin, pea; Legumin, vetch; Vicilin; Vignin; Glycinin.*—The results given in these tables require discussion together, as they depend largely on the nature of the preparations used, as well as on the nature of the proteins contained in the seeds, peas, vetches, cow-peas, and soy-beans from which they were obtained. Legumin is the chief protein constituent of the first two seeds, preparations from each of which are so nearly alike in all respects that a most extensive and rigid comparison has as yet revealed no positive difference between them.* It is not surprising therefore to find that guinea-pigs sensitized with pea legumin showed marked anaphylaxis reactions when subsequently injected with vetch legumin. Conversely when sensitized with vetch legumin reactions were obtained with pea legumin, which, however, were usually much less severe, although approximately as strong as those obtained by sensitizing and intoxicating with pea legumin itself.

Besides legumin, the pea contains another protein of slightly different properties and composition to which the name vicilin has been given. Vicilin, which has not been found in the seeds of the vetch, is so difficult to separate from legumin that it is probable that no preparations of either one of these proteins from the pea have ever been made which did not contain enough of the other to cause the extremely delicate anaphylaxis reaction. The preparations of pea legumin and vicilin used for our experiments were made by extensive fractional precipitation with $(NH_4)_2SO_4$ and represent products which, according to our past experience, should be very pure, especially in respect to the separation of the one protein from the other. It is, therefore, striking to find that four animals sensitized with pea legumin promptly died when treated with vicilin and that of four sensitized with vicilin and treated with pea legumin three died, though less quickly, and one became severely ill. That the reaction between vicilin and pea legumin, when tested either way, was so much more severe than between pea legumin and pea legumin (in our experiments no fatal reactions were obtained with the latter combination) suggests that the extensive fractional precipitation with $(NH_4)_2SO_4$ has contributed to the result in some way that deserves further careful study. A similar result has been reported by Gay and Adler[57] with preparations of serum proteins made by fractional precipitation with $(NH_4)_2SO_4$, and one of us (W.) has obtained results with the proteins of egg white which suggest that in natural protein mixtures there may be present substances which decrease the anaphylactic activity of some of the proteins.

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* Cf. Osborne, "Die Pflanzenproteine," *Ergebnisse der Physiol.*, 1910, 10, p. 119.

When vicilin was used for sensitizing, a distinct reaction was obtained when vetch legumin was subsequently injected, but much less severe than when pea legumin was used. On the other hand, when vetch legumin was used for sensitizing and pea vicilin for intoxicating, the results were fatal in three cases, severe in one, and moderate in four, with no effective protection conferred against the vetch legumin. When vicilin was used for sensitizing, a distinct and often severe, but in no case fatal, reaction was obtained.

The preparation of vetch legumin was made by repeated precipitation from NaCl solutions and was not subjected to fractional precipitation with $(NH_4)_2SO_4$. It is difficult to find a satisfactory explanation of the results obtained with these three proteins unless the fractional precipitation with $(NH_4)_2SO_4$ has involved some factor which in some way concerns the anaphylaxis reaction. It is our intention to investigate this matter further, for it would seem as if we had here a clue which will lead to a better understanding of this important reaction.

Vignin, the principal protein of the cow-pea, closely resembles legumin in properties, composition, and relative proportion of the decomposition products which it yields on hydrolysis. The slight differences between these proteins are, however, sufficient to characterize them as chemically distinct substances. The preparation of vignin used in these experiments was made by repeated precipitation from NaCl solutions and was not subjected to fractionation with $(NH_4)_2SO_4$.

Unlike legumin, from the pea or vetch, vignin gave a very strong anaphylaxis reaction. Animals sensitized with vignin reacted in several cases with vetch legumin, but only doubtfully with pea legumin. Animals sensitized with vetch legumin reacted to vignin in some cases, in others not at all, and acquired only partial protection. Those sensitized with pea legumin gave, at the most, only doubtful reactions and showed no protection, the second injection of pea legumin reacting more strongly even than in those experiments where the second dose of pea legumin followed the first without the intervening dose of vignin. It is possible that the fractionation of the pea legumin with $(NH_4)_2SO_4$ had an influence in this case, but further experiments are necessary before definite conclusions can be drawn. Experiments with vicilin and vignin, which have not been tried, should also be made. Guinea-pigs sensitized with vignin did not react with glycinin, which is a protein of very similar properties and composition, obtained from the soy-bean. On the other hand, those sensitized with glycinin in most cases reacted to a considerable extent with vignin, but without pronounced protection.

The seeds of the pea, vetch, cow-pea, and soy-bean all contain small amount of an albumin-like protein (legumelin) which, so far as is now known, may be identical in each of these seeds. Sufficient data are not, however, available to indicate the real relation of the preparations of this protein from the different seeds. It is possible that the interreactions of legumin, vicilin, vignin, and vicilin may be connected with slight contaminations of our preparations with traces of legumelin, and that the difference in behavior of the preparations of legumin from the pea and vetch may be due to a more complete separation from legumelin by the fractional precipitation with $(NH_4)_2SO_4$ employed in making the pea legumin.

Between proteins of markedly different structure neither vignin nor glycinin showed any reaction, e.g., excelsin+vignin, or hordein+vignin; glycinin+excelsin, or excelsin+glycinin.

Table 12. *Gliadin, wheat.*—The anaphylactic relations of gliadin from the wheat and that from the rye kernel offer a special interest, for preparations of these two proteins from each of these seeds are so nearly alike that the only marked difference yet noted is a slight difference in the specific rotation, observed in but a single case, and, therefore, requiring confirmation before it can be accepted as final.

It is interesting to note that our experiments show that sensitizing with preparations from one seed yield as severe symptoms on afterward injecting those from the other seed as when the same preparation was used for each injection. Owing to the similar appearance of rye and wheat and their frequent cultivation in neighboring fields it is possible that the flour from which our preparations were obtained was not entirely free from seeds of the other species. In order to exclude the effect of such contamination the amount of the sensitizing dose was varied widely without giving results which indicated that any contamination existed which was sufficient to affect the results; furthermore, the efficient protection exhibited by animals which had recovered from intoxication with the heterologous gliadins is conclusive proof that we are here dealing with a sensitization with one protein, and not with a mixture of two distinct proteins from wheat and rye respectively.

It is also interesting to find that between gliadin and the somewhat similar alcohol-soluble zein from maize, no reaction occurs.

Table 13. *Hordein.*—Hordein, the alcohol-soluble protein of barley, is similar to gliadin in solubility and the proportion of its products of hydrolysis. The anaphylactic relations of these two proteins have not yet been determined. Like gliadin, hordein gave no reaction with the alcohol-soluble zein nor with the very dissimilar vignin.

Table 14. *Zein.*—The most important fact shown by the results given in this table is the effect of diet on the anaphylaxis reaction. With guinea-pigs which had never been fed with corn a promptly fatal result was obtained in most cases and severe results, with but two exceptions, in the others, whereas those animals that were raised on corn diet gave no reaction whatever. It may be noticed that the toxic dose in the first eight cases was twice that given in all the other experiments here cited, but numerous control experiments show that this large dose of zein does not itself cause noticeable intoxication (see expts. 35 and 36).

c) Classification of Vegetable Proteins According to Their Specificity.

On the basis of these experiments we may, provisionally, classify the vegetable proteins investigated according to their biological reactions, as follows. (The protein first named was used for sensitizing, and the second for intoxicating.)

A. SPECIFIC.

Castor-bean globulin+edestin
Squash-seed globulin+flax-seed globulin
" " " +castor-bean globulin
Flax-seed globulin+squash-seed globulin
Edestin+castor-bean globulin
" +squash-seed globulin
Excelsin+vignin
" +glycinin
" +squash-seed globulin
" +castor-bean globulin
Legumin, vetch+glycinin, apparently some protection.
Vignin+legumin, pea
" +glycinin
" +hordein
Glycinin+excelsin
" +vicilin
Gliadin+zein
Zein+gliadin
" +hordein, apparently some protection.
" +castor-bean globulin
Hordein+zein
" +vignin

B. NON-SPECIFIC.

Legumin, pea+legumin, vetch, 3 results fatal, 1 severe, 3 moderate, and 2 doubtful; protection distinct.
" " +vicilin, pea, all results fatal; protection not ascertained.

Legumin, vetch+legumin, pea,	1 result moderate, 1 slight, and 4 doubtful; distinct protection.
“ “ +vicilin, pea,	3 results fatal, 1 severe, 4 moderate; no protection.
Vicilin+legumin, vetch,	4 results severe, 1 slight, 1 doubtful, 2 none; no pronounced protection.
“ +legumin, pea,	3 results fatal, 1 severe; protection not determined.
Vignin+legumin, vetch,	2 results fatal, 1 severe, 1 doubtful, 2 none; no protection.
Gliadin, wheat+gliadin, rye,	4 results fatal, 3 severe, 1 moderate; some protection.
“ rye+gliadin, wheat,	2 results fatal, 4 severe, 1 none; distinct protection.

c. DOUBTFUL.

Castor-bean globulin+flax-seed globulin,	2 results fatal, 5 slight, 2 doubtful, 1 none; no protection.
“ “ “ +squash-seed globulin,	4 results doubtful; no protection.
Squash-seed globulin+excelsin,	1 result fatal, 6 moderate, 4 doubtful; no protection.
“ “ “ +edestin,	4 results slight, 4 none; no protection.
Flax-seed globulin+castor-bean globulin,	3 slight, 1 moderate; distinct protection.
Excelsin+edestin,	4 results doubtful; no protection.
Edestin+excelsin,	3 results moderate, 1 slight, 4 none; doubtful protection.
Legumin, pea+vignin,	8 results doubtful; no protection.
“ vetch+vignin,	2 results severe, 1 moderate, 1 slight, 4 none; no pronounced protection.
Glycinin+vignin,	3 results severe, 2 moderate, 2 slight, 1 none; no protection.
“ +vicilin,	1 result moderate, 3 doubtful; no protection.

d) Conclusions.

The only positive reactions between proteins from different seeds occurred between the legumins from the pea and vetch, vicilin from the pea and legumin from the vetch, the gliadins from wheat and rye, and vignin and vetch legumin. The reaction between the two latter proteins was rendered doubtful by some of the experiments in which doubtful or negative reactions were obtained and by the absence of any protection. Preparations of legumin from the pea and vetch are very similar, if not identical, as are also those of gliadin from wheat and rye; while legumin and vignin, though much alike both in their properties and the proportion of their decomposition products, are evidently not identical. The reactions between the legumins and vicilin are probably due to an incomplete separation of these two proteins from the other proteins of the seeds.

All of the doubtful reactions were obtained between proteins having very similar properties and yielding similar proportions of decomposition products. However, in several cases the reactions were wholly specific between proteins which were even more nearly alike according to our present knowledge.

It would seem from such data as we have thus far obtained that the chemical constitution of the protein was more concerned in the anaphylaxis reaction than the biological relations of the seeds from which they originate, but more evidence is required before this conclusion can be definitely accepted.

The results obtained with the preparations of legumin from the pea and those from the vetch, and also of vicilin, make it important to determine the effect of extensive fractionation with $(NH_4)_2SO_4$ on the outcome of anaphylaxis experiments.

10. SUMMARY.

Up to the present time nearly all the studies of the biological reactions of proteins have been made with mixtures of proteins such as exist in the fluids which occur naturally in the animal body, or extracts containing soluble constituents of animal or plant tissues. In order to determine the chemical relations of the biological reactions it is fundamentally important to use preparations of proteins of the highest possible purity, and especially those whose composition is known, as far as our analytic measures permit.

Since technical difficulties at present render an exact isolation of animal proteins an almost impossible task, it would seem best to undertake the study of the chemical principles and processes of the biological reactions with preparations of vegetable proteins, many of which can be isolated in a relatively pure condition. This paper gives the results of a preliminary survey of the field.

A review of the literature shows that very few investigations have been made of the biological reactions of vegetable material, and that most of these have been made with substances of indefinite character. There is already sufficient evidence to indicate that the specificity of the anaphylaxis reaction corresponds to that of the other biological reactions; and that this reaction involves, in all probability, essentially the same sort of reaction to a foreign protein antigen as do the precipitin and complement fixation reactions. Because a large amount of data can be obtained in a comparatively short time, we chose the anaphylaxis reaction for our first orienting experiments with preparations of some of the vegetable proteins, whose chemical composition has already been investigated by analytic methods.

Experiments have been performed with globulin from the castor bean (*Ricinus communis*), flax seed (*Linum usitatissimum*), and squash seed (*Cucurbita maxima);* with edestin from hemp seed (*Cannabis sativa*), excelsin from the Brazil nut (*Bertholletia excelsa);* with proteins from the cocoanut (*Cocos nucifera*), legumin and vicilin from peas (*Pisum sativum*), legumin from the vetch (*Vicia sativa*), vignin from the cow-pea (*Vigna sinensis*), glycinin from soy-bean (*Soja hispida*), gliadin from wheat flour (*Triticum vulgare*) and rye flour (*Secale cereale*), hordein from barley flour (*Hordeum vulgare*), and zein from maize (*Zea mays*).

It has been found that all these proteins cause typical anaphylaxis reactions in sensitized animals, with all features essentially the same as when serum and other animal materials containing soluble proteins are used. The minimum doses which produce sensitization and the time of incubation are about the same as with animal proteins, but as a rule the symptoms are of somewhat slower onset and less stormy course than are those obtained with foreign sera, and the minimum intoxicating doses are larger. There are also considerable differences in the toxicity of the several vegetable proteins to sensitized animals, but the reasons for these differences have not yet been investigated. The most toxic proteins, as measured by the frequency of severe and fatal reactions, were the globulin of the squash seed, vignin, excelsin, and castor-bean globulin, which usually caused death when given in 0.1 gm. doses to properly sensitized animals. Edestin caused the least severe reactions of any of the proteins, while hordein and glycinin seldom caused fatal reactions; nevertheless the minimum sensitizing and intoxicating doses of edestin and squash-seed globulin are essentially the same.

The influence of the food of the guinea-pigs upon the anaphylaxis reaction is of particular importance in experiments with vegetable proteins, since the natural food of guinea-pigs is vegetable. Experiments showed that continuous feeding with a vegetable protein rendered guinea-pigs immune to this protein, so that they could not be sensitized to it. Although brief feeding with animal proteins (cow's milk, foreign sera, egg albumin) renders the animal sensitive to the corresponding animal protein, probably sufficiently protracted feeding with animal proteins will likewise confer immunity. The sensitization through feeding is specific for the protein fed, showing that during the processes preceding and including absorption of the food protein, no change takes place which robs it entirely of its biological specificity.

Marked specificity of reaction was shown, within certain limits, by the vegetable proteins employed. The close similarity, if not identity, of the legumins of the pea and vetch was shown by the interreaction of these proteins, and the close relation to vicilin from the pea was also indicated. The near relation or probable identity of the gliadins from wheat and rye was also shown. In some instances doubtful results were obtained, for example with some guinea-pigs, castor-bean globulin and flax-seed globulin interacted strongly, while with others similarly treated no reactions were obtained. The significance of the results of experiments upon specificity, with particular reference to the relation of chemical composition to the specificity of the biological reactions, is discussed.

The results obtained indicate the importance of future investigations along several lines which are now being followed as rapidy and thoroughly as possible.

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